

EXPERIENTIA



REVUE MENSUELLE DES SCIENCES PURES ET APPLIQUÉES
MONATSSCHRIFT FÜR DAS GESAMTE GEBIET DER NATURWISSENSCHAFT
RIVISTA MENSILE DI SCIENZE PURE E APPLICATE
MONTHLY JOURNAL OF PURE AND APPLIED SCIENCE

Editeurs:

P. HUBER · R. MATTHEY · A. v. MURALT · L. RUZICKA
Basel Lausanne Bern Zürich

Redactor: H. MISLIN, Basel-Mainz

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Dernier délai d'admission pour les manuscrits: 2 mois avant la parution. Les auteurs recevront gratuitement, s'ils le désirent, 75 tirés à part de format 14,5 sur 21 cm, sans couverture. Pour le prix d'un nombre plus grand et pour la couverture s'adresser à l'éditeur. Les tirages à part doivent être commandés *avant* l'impression du périodique.

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L'EXPERIENTIA est imprimée en Suisse.

*Editions Birkhäuser S. A., Bâle 10 (Suisse), Elisabethenstrasse 19
Adresse télégraphique: Edita Bâle*

Die EXPERIENTIA erscheint am 15. jedes Monats und kann im In- und Auslande durch jede Buchhandlung oder direkt vom Verlag bezogen werden. In Algerien, Belgien, Dänemark, Finnland, Frankreich, Italien, Luxemburg, Marokko, Norwegen, Österreich, Portugal, Schweden nehmen auch die Postämter Bestellungen entgegen. Der Abonnementspreis beträgt in der Schweiz Fr. 44.-, im Ausland sFr. 56.-, (DM 56.-).

Alle Zuschriften an die Redaktion sind ausschliesslich an den Verlag zu richten. Redaktionsschluss 2 Monate vor Erscheinungsdatum.

Die Autoren erhalten auf Wunsch 75 Gratisseparata im Format 14,5 x 21 cm, ohne Umschlag. Die Kosten für weitere Separata und für Umschläge sind beim Verlag zu erfragen. Separata sind *vor* dem Druck der Zeitschrift zu bestellen.

Insertionspreise: $\frac{1}{2}$ Seite Fr. 220.-, $\frac{1}{4}$ Seite Fr. 132.-, $\frac{1}{8}$ Seite Fr. 77.-; für Vorzugsseiten besondere Vereinbarung. Inseratenannahme durch den Verlag.

Die EXPERIENTIA wird in der Schweiz gedruckt.

*Birkhäuser Verlag AG., Basel 10 (Schweiz), Elisabethenstrasse 19
Telegraphadresse: Edita Basel*

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Gli autori ricevono, su desiderio, 75 estratti del formato 14,5 x 21 cm, senza copertina. Il prezzo degli estratti in più e della copertina viene indovato, su richiesta, dalla casa editrice. Gli estratti vanno ordinati *prima* della stampa della Rivista.

Prezzi per annunci: $\frac{1}{2}$ pag. fr. 220.-, $\frac{1}{4}$ pag. fr. 132.-, $\frac{1}{8}$ pag. fr. 77.-; per pagine speciali, accordi da stabilire. Gli annunci sono da inviare alla casa editrice.

EXPERIENTIA si stampa in Svizzera.

*Casa editrice Birkhäuser S. A., Basilea 10 (Svizzera), Elisabethenstr. 19
Indirizzo telegrammi: Edita Basilea*

EXPERIENTIA is published on the 15th of every month and can be obtained in any country through booksellers or from the publishers. The annual subscription price by inland mail is fr. 44.-; other countries fr. 56.-. Prices in Swiss currency. All communications to the editors should be addressed to the publishers. All manuscripts for publication in a given number must be in the hands of the publishers 2 months before the publication.

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*Published by Birkhäuser Ltd., Basle 10 (Switzerland), Elisabethenstr. 19
Telegrams: Edita Basel*

Printed in Switzerland / Birkhäuser AG. Basel

Verzeichnis der Inserenten - Liste des annonceurs - List of Advertisers - Experientia XIII/5

Birkhäuser Verlag, Basel

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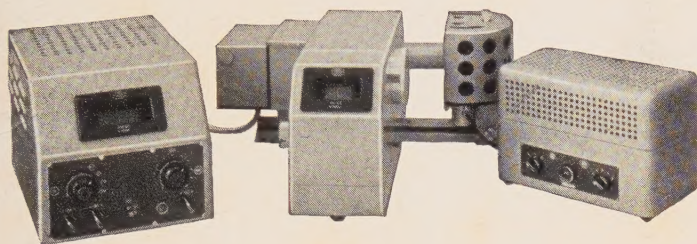
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Genetical Effects of Radiation and Chemicals

By CHARLOTTE AUERBACH, Edinburgh*

Editor's Note. The progress of nuclear physics and nuclear chemistry has led up to the present atomic age. Now the science of genetics has shown that the ionising rays which are set free in atom disintegration processes, are capable of changing the genes and thus leading to irreversible damage to the hereditary material. The two following contributions by well-known experts of radiation genetics and chemico-genetics stress implicitly the responsibilities which arise from the inseparable connection between the effects of atomic energy and genetics, as also between chemicals and genetics.

During the last few years civilized man has become radiation conscious. Most people realise that exposure to ionizing radiation—be it from atom-bomb fall out, from radioactive waste-products, from X-rays or any other source—is dangerous to the individual and his progeny; but about the nature and degree of this danger only few are in a position to form an opinion. It must be admitted that at present even the expert can do no more than determine the limits of probability within which various harmful effects of radiation may take place, but this does nevertheless eliminate the two extreme attitudes: exaggerated anxiety, usually based on misunderstood press articles or lectures, and facile optimism, caused by ignorance of the biological facts and principles involved. It is the aim of the present article to acquaint the reader with this biological background insofar as it relates to genetical damage, i.e. damage incurred by the gametes of the exposed individual and manifesting itself in the progeny. For this purpose, it will first be necessary to describe briefly the genetic material and its functioning.

The genetic material.—Ionizing radiations affect chemical substances, in particular organic molecules. If the effects are on the molecules of the genetic material, inherited changes are produced. In all higher organisms the genetic material is organised into microscopically visible structures, called chromosomes. These are present in the nucleus of every cell and become visible when the cell divides. Numbers and shapes of chromosomes are a fixed property of a given species; thus maize cells contain 20 chromosomes, mouse cells 40, etc. In a sexually produced organism the chromosomes consist of two sets, one of which is derived from the father, the other from the mother. Every chromosome in the

paternal set is matched by a similar chromosome in the maternal set. The only exceptions are the sex-chromosomes which determine the sex of the individual. In many species these chromosomes form an unequal pair in one sex. In mammals, the female has an accurately matched pair of chromosomes; they are called X-chromosome. The male has only one X-chromosome. Its partner is a so-called Y-chromosome, which differs from the X in size and shape and carries very few genes.

The most remarkable property of the chromosomes is their capacity for replication. In between any two cell divisions, an exact replica of each chromosome is laid down close to the original; when division takes place, original and copy separate and go into different cells. As a result, all cells which arise by division from the same ancestral cell contain the same genetic material. There is one important exception to this rule: in the divisions which precede the formation of gametes, partner chromosomes pair and the two members of a pair go into different cells, so that the gametes contain only one set of chromosomes.

Breeding experiments, corroborated by cytological findings, have firmly established that each chromosome is differentiated lengthwise into many minute segments—called genes—which are characterized by their various effects on development. Genes, interacting with environment, determine the multitude of traits which go into the making of an individual: its outward appearance, its physiological functions, its disease resistance, its ability to perform biosyntheses, its behaviour and mental endowment.

Allelomorphs.—Many or perhaps all genes within a species exist in a variety of forms, called allelomorphs. Allelomorphs occupy corresponding loci on corresponding chromosomes. They also influence the same developmental process, but they do so in ways that may differ qualitatively or quantitatively. A series of allelomorphic genes underlies the differentiation of mankind into the four bloodgroups of the ABO system; black and red cats differ in the possession of one or the other allelomorph of a gene concerned with the formation of coat colour; small and large strains within a species carry different allelomorphs, often of a number of genes determining growth rates. When a gene produ-

* Institute of Animal Genetics, Edinburgh.

ces an abnormality of, say, the skeleton, we infer the existence of a normal allelomorph which is concerned with the normal development of, in this instance, the skeleton.

As the chromosomes are present in pairs, so are the genes which they carry. Every sexually produced individual has a duplicate outfit of genes, one set of paternal, the other of maternal origin. An exception is formed by those genes in the X-chromosome for which the Y carries no allelomorphs. These genes are called sex-linked, and the XY sex has only one set of them. In the domestic cat, the allelomorphs for black or red coat colour are sex-linked. For a tomcat there are only two possibilities in regard to these genes: he may have a 'red' gene and a red coat, or a 'black' gene and a black coat. A female cat has three possibilities: she may have two 'black' genes and be a black cat; she may have two 'red' genes and be a red cat; she may also have one 'red' and one 'black' gene, in which case she is a tortoiseshell. Black and red females are said to be *homozygous* for the 'black' or 'red' allelomorphs respectively; a tortoiseshell female is said to be *heterozygous* for the 'black' and 'red' allelomorphs. It will be seen that a mammalian male cannot be heterozygous for a sex-linked gene, although—just like a female—he may be homozygous or heterozygous for any other gene.

Heterozygosity does not usually result in individuals which, like the tortoiseshell cat, display a mosaic of the two contrasting gene effects. In fact, this is a rare situation. Often the heterozygote is intermediate in appearance between the homozygotes: a mulatto is heterozygous for two or more pairs of allelomorphs which in his parents determine black and white skin colour respectively. There are also many cases in which only one allelomorph produces a noticeable effect in the heterozygote. The gene for albinism interferes with the formation of pigment in skin, hair and iris; its normal allelomorph controls a biochemical process which is essential for pigment formation. Mice or rabbits which are homozygous for the albino gene have white fur and red eyes; heterozygotes are indistinguishable from coloured homozygotes for the normal allelomorph. In such cases, we call the allelomorph which is effective in the heterozygote *dominant* over the other, *recessive* allelomorph. Many harmful recessives may be carried in heterozygous condition by perfectly healthy individuals. Congenital deafmutism is often due to a recessive gene. In families in which such a gene occurs some individuals are deaf because they are homozygous for it; many more are outwardly normal heterozygotes carrying the gene for deafness together with its normal allelomorph. When these persons produce gametes, the two partner genes separate and half the gametes contain the gene for deafness. When two heterozygotes marry, some of the children receive the harmful recessive from both parents and

are deaf. The chance that marriage partners may be heterozygous for the same kind of harmful gene is higher for relatives than for unrelated persons; for relatives may have received this gene from a common ancestor. This explains why cousin marriages result in defective children more frequently than do marriages between people not so closely related.

All possible gradations are found between genes which are completely dominant over their allelomorphic partners and others which produce intermediate effects in the heterozygote. Refined methods of investigation often allow the detection of a seemingly recessive gene in the heterozygote. In the fly *Drosophila*, as in man, the male has the genetical constitution XY and carries only one set of sex-linked genes, while the female is XX and carries genes in duplicate. Many sex-linked genes are known in *Drosophila* which harm or kill the male (so-called sex-linked deleterious or lethal genes), but can be carried in heterozygous condition by normal healthy females. In two independent investigations the reproductive ability of females which carried a sex-linked lethal in heterozygous condition was compared with that of normal homozygous sisters; in both cases, the average performance of the heterozygotes was slightly, but significantly, inferior to that of the normal homozygotes. The only reason for doing these tests on *sex-linked* lethals was one of convenience; it is highly probable that the result would have been similar if, instead, heterozygotes for lethals on another chromosome would have been compared with normal homozygotes. The possibility that in man also, genes which are drastically harmful to the homozygote—or to a man carrying them on his single X-chromosome—may be slightly disabling in heterozygotes is an important point to consider in the assessment of genetical radiation damage.

The action of radiation on the genetic material.—Radiation produces two types of change in the genetic material, both of which occur also spontaneously in nature, but at very much lower frequencies: chromosome breaks and mutations.

CHROMOSOME BREAKAGE.—In suitable material, e.g. plant cells with large chromosomes, chromosome breakage and the secondary processes resulting from it can be microscopically demonstrated. Once a chromosome break has been produced, it may have one of three possible fates:—(1) It may remain open, and the cell now contains a fragmented chromosome. Such a cell survives as long as it does not divide, but for reasons which are not yet fully understood it usually dies in the attempt at division. Even if the cell manages to go through one or a few divisions, the chromosome fragments are apt to be lost on the way, so that cells are produced which no longer possess all the genes necessary for survival. Thus, by various means, fragmented chromosomes kill dividing cells. (2) The broken pieces may heal together in the old way. This usually

leaves the cell unharmed. (3) The broken pieces may join in a novel way, resulting in a 'chromosome rearrangement'. Two important types of rearrangement are 'deficiencies' and 'translocations'. A deficiency arises when a chromosome has been broken in two spots and the two terminal pieces fuse, leaving the middle piece to be lost at one of the next divisions. A small deficiency may not be lethal to the cell because the function of the missing genes may be adequately performed by their allelomorphs on the partner chromosome. The larger the deficiency, the larger the chance that among the lost genes there are one or more which have to be present in duplicate for survival; large deficiencies are therefore usually lethal. A translocation is an exchange of pieces between two broken chromosomes. This may or may not result in death of the cell, depending on the way in which the broken pieces have joined. However, even translocations which are compatible with life interfere with the formation of normal gametes; for when the chromosomes pair in preparation for gamete formation the translocated pieces have difficulties in finding partners, and gametes are produced which no longer possess one and only one chromosome and gene of each kind. Individuals which carry a translocation in heterozygous conditions have therefore greatly reduced fertility.

MUTATIONS.—Mutations are changes which arise suddenly and produce variation which is inherited according to MENDEL's fundamental laws of heredity. It is true that changes which fulfil these requirements may also be brought about by chromosome rearrangements. Thus translocations produce inherited semi-sterility, deficiencies may produce all kinds of inherited abnormalities, and the mere fact that a chromosome rearrangement creates new neighbourhood relationships between genes may affect the functioning of the genes by what is called 'position effect'. There are geneticists who attribute all hereditary changes to chromosome rearrangements. In cases where microscopical and genetical techniques fail to show any chromosomal abnormality, it is postulated that the rearrangement is too small for detection. This theory is difficult to disprove, but the majority of geneticists believe that in addition to chromosome rearrangements there exists a class of hereditary changes which is due to chemical changes in individual genes. Theoretically, the question whether such true gene mutations exist is of great interest; for practical purposes it is convenient and sufficient to distinguish between large, easily detectable chromosome rearrangements and changes affecting only one or a few neighbouring genes, and reserve the term 'mutation' for this latter class.

Mutations may affect every aspect of the living individual: its structure, its physiology, its cellular biochemistry, its mental faculties and behaviour. They may be lethal like deficiencies; they may cause inferti-

lity like translocations. The vast majority of mutations are harmful—very few indeed are beneficial. This may seem a contradiction if one considers that without mutation there could have been no evolution. The contradiction resolves itself when one realises that a living species in its present habitat is a product of evolution, and, as such, is finely adapted to its environment and mode of life. Mutations are random events which are likely to disturb the balance between the organism and its environment, and between the multitude of subtly interwoven life processes within the organism.

The spread of mutations through a population.—The speed with which a newly arisen mutation becomes manifest and the manner in which mutated individuals are distributed over the succeeding generations depends on whether the mutated gene is dominant or recessive, sex-linked or unrelated to sex. A dominant mutation becomes manifest in the first individual carrying the mutated gene. A recessive mutation may be carried latent for many generations until mutated individuals appear among the offspring of two heterozygotes. A recessive sex-linked mutation has a better chance of manifesting itself; although, in a species like man or *Drosophila*, it may remain hidden for several generations in heterozygous females, it shows up in the first male which carries it on its single X-chromosome.

Mutation is a process from which there is no recovery. The replicas of a mutated gene or a rearranged chromosome are a similarly mutated gene or a similarly rearranged chromosome. Although the individual cannot rid itself of its harmful mutated genes and chromosome rearrangements, the population as a whole can do so and must do so if it is not to die out as a result of the harmful mutations which very slowly, but inexorably, accumulate in every living species. Every time a genetically afflicted individual dies childless before the end of its reproductive life, the genes which caused this untimely death are removed from circulation among the next generation. The same happens when a gene, by curtailing fertility, reduces the number of offspring to which it might have been transmitted. Eventually, a balance is established between the rate at which harmful genes arise *de novo* through mutation and the rate at which they are lost through early death or infertility. In a population which has reached this equilibrium, every kind of mutated gene is present in a frequency which is determined by the rate at which it arises and the rate at which it is discarded. It has been estimated that in the present human population, every individual carries at least 8 harmful genes.

Quantitative radiation genetics.—It is not sufficient to know what-kind of genetical changes are to be expected from exposure of the germ cells to radiation. One also wants to know the absolute and relative frequencies with which the various types of effect are produced by a given dose and kind of radiation. For some

genetically well-analyzed species, in particular *Drosophila* and maize, such predictions can be made with reasonable certainty; applied to our own species, most of them have a very large margin of uncertainty. There are, however, certain general quantitative rules which hold good in all investigated species and which almost certainly are applicable also to man. These are rules relating the overall frequencies of certain broad classes of genetical change to dose and type of radiation.

I. Dose-effect relations

Mutations.—(1) The frequency of gene mutations, including that of very small rearrangements, is directly proportional to dose as measured in roentgen(r) units. This rule has been found to hold down to the lowest dose tested (25r), and there is no reason to assume that it will not apply to even lower doses.

(2) The frequency of mutations is determined by the total amount of r units received by the gonads and is independent, within wide limits, of wavelength and intensity of delivery, i.e. the reciprocal of the time over which a given dose is spread. It also remains unchanged when a given dose is administered in several fractions, separated by periods of rest, instead of in one continuous exposure.

From these observations the conclusion has been drawn that a gene mutation is the result of a single ionization—or of a small cluster of ionizations—which takes place inside a gene or very close to it. This interpretation has remained essentially correct, although we have come to realise that very often short-chain chemical processes are intercalated between ionization and mutation. It leads to conclusions which, although they are implicit already in the statements (1) and (2), are so important that they will be re-stated explicitly as—

(3) There is no lower threshold to the production of mutations by ionizing radiation. Every dose produces mutations in proportion to its magnitude.

(4) The number of mutations which a given dose produces in the germ cells of an individual is independent of how the exposure is spread in time. The same number of mutations is produced in the germ cells of a woman who, at the age of 24, receives one exposure of 250 r to her gonads as in those of a woman who, from the moment of conception to the age of 24, was exposed to chronic irradiation amounting to a yearly gonad dose of 10 r.

(5) The number of mutations which a given dose produces in the germ cells of a population is independent of how the exposures are distributed over individuals. 250 r received by the gonads of one individual produce the same number of mutations as 0.1 r received by the gonads of 2,500 individuals.

To be strictly accurate, (4) and (5) would need to be corrected for differences in sensitivity between various types and stages of germ cell, but the correction

factors are probably never large and cannot obscure the essential features of these important conclusions.

Chromosome breaks and rearrangements.—(6) The frequency at which chromosome breaks originate increases linearly with dose and follows the rules which have been listed for mutations.

(7) Large chromosome rearrangements, such as translocations or large deficiencies, behave differently. A chromosome rearrangement is a secondary effect of chromosome breakage; it requires the presence in the same cell of two chromosome breaks. Neutrons may produce the two required breaks along the same track of protons; neutron induced rearrangements are, therefore, directly proportional to dose. With less densely ionizing radiation, in particular X-rays and gamma-rays, the two breaks have to be produced independently, and the frequency of rearrangements increases approximately as the square of the dose; approximately and not accurately, because of complications on the biological level, such as inviability of cells in which many breaks have occurred. Because of this dose-effect relationship, rearrangements become negligibly rare at low doses.

(8) The frequency of rearrangements is not, like that of mutations, independent of the intensity at which a given dose is administered or of the choice between continuous or fractionated exposure. At low intensities and after fractionation, rearrangements are less frequent than would be expected from the overall dose. This is so because chromosome breaks remain open and rejoinable for a limited time only. When the intensity of radiation is low or when the dose is fractionated, the second break will often be produced when the first is no longer available for the formation of a rearrangement. In certain types of cell, notably in mature spermatozoa, chromosome breaks can be stored indefinitely without losing their rejoining ability; the frequency of X-ray induced rearrangements in spermatozoa is, therefore, independent of radiation intensity and degree of fractionation of dose.

II. The relative frequencies of different types of genetical effect

Physical as well as biological factors influence the proportions with which different types of genetical effect appear after irradiation. Thus, at high doses, rearrangements are relatively more frequent than at low ones because the dose-effect curve is steeper for rearrangements than for mutations. All types of rearrangement are very rare after irradiation of early germ cells, spermatogonia or oogonia. In the mouse, with 20 chromosome pairs, translocations form a larger proportion of all rearrangements than in *Drosophila* with 4 chromosome pairs. In *Drosophila*, X-rays produce changes which by all known criteria are true gene mutations; in maize, X-rays seem to act only destructively on the chromosomes, and, on closer analysis, all

apparent gene mutations were found to be small deficiencies.

Yet, there is one relation which we may expect to hold for all organisms, including man. This is the vast preponderance of harmful and lethal mutations. The theoretical reasons for expecting just such a preponderance have been briefly mentioned before. In practice, it has been found in every tested species. In the best analysed species, *Drosophila*, by far the largest class of mutations consists of harmful genes which, without producing any visible abnormality, lower viability and reduce fertility. The next largest class is recessive lethal mutations, i.e. mutations which kill homozygotes (or males carrying such a mutated gene on their single X-chromosome), while heterozygotes are normal or only slightly inferior to normals. Visible mutations, with only slight or no harmful effect, are much rarer, and the majority of them are recessive. The relative scarcity of dominant as compared with recessive visibles is a general observation, made also for chemically produced and naturally occurring mutations. This is not the place to discuss the various hypotheses which have been put forward in explanation.

III. Sensitivity differences between germ cells

It has already been mentioned that not all types of germ cells are equally sensitive to the mutagenic action of irradiation. In the mouse as well as in *Drosophila*, a given X-ray dose produces more mutations in spermatids than in any other stage of male spermatogenesis; in both species, mature spermatozoa are somewhat less sensitive than spermatids, and spermatogonia are the least sensitive stage. This parallelism between two widely divergent species suggests a fundamental sensitivity pattern of the animal testis. It seems reasonable to infer the existence of a similar pattern in the human testis. Information on the production of mutations in female germ cells is still too incomplete to warrant generalisations.

More important than differences in sensitivity between germ cells of the same species is the possibility that the frequencies of mutations which a given radiation dose produces in a given type of germ cell may differ between species; for at present our only means of arriving at some quantitative estimate of radiation damage to the human species consists in extrapolation from lower forms of life, especially *Drosophila*. It is, therefore, of the utmost importance that mouse germ cells have been found to be considerably more sensitive to the genetical effects of X-rays than *Drosophila* germ cells. It is certain that a given X-ray dose produces many more chromosome breaks and translocations in the mouse than in *Drosophila*, but this might be simply a consequence of there being more chromosome material to be broken. In addition, however, there is evidence that, gene for gene, a dose of X-rays produces more mutations in the mouse than in *Drosophila*. The

magnitude of this difference is still being examined; its existence can hardly be doubted. It seems probable that in this, as in all other respect, man is closer to the mouse than to *Drosophila*.

Genetical effects of radiation on human populations. Let us first consider persons who have received a fairly high dose of radiation to their gonads, such as the atom bomb survivors in Hiroshima and Nagasaki, or women whose ovaries have been exposed to a high X-ray dose. There is a popular idea that all kinds of monstrosities may turn up among the children of such persons. This is entirely wrong, for dominant mutations which might produce such abnormalities after irradiation of one of the parents are the rarest type of mutation. Recessive mutations with visible effect on the homozygote are much more frequent, but for these to become manifest in the children of irradiated parents, the same mutation would have to be produced in both spermatozoon and ovum, and the chance for this to happen is infinitesimally small. It is, therefore, not surprising that there was no significant increase in the incidence of freaks or monstrosities among children of persons who survived the Japanese atom bomb explosion, and that persons whose gonads have been X-rayed may have healthy and normal children. These findings cannot be taken as evidence that radiation has not caused genetical damage of a less immediately detectable nature.

Among women who were pregnant at the time of the atom bomb explosion, a number gave birth to physically or mentally defective children. This, however, was not a genetical effect of the radiation, but a direct effect on the developing embryo, comparable to an X-ray burn which develops after exposure of the skin. Should the affected children grow up to reproduce they will not transmit the damage which they themselves suffered. It is true that they may transmit genuine genetical changes—mutations or chromosome rearrangements—which were induced in their foetal gonads; but, if so, there will be no resemblance—except perhaps a fortuitous one—between these genetically caused abnormalities and the original radiation damage. Thus, irradiation of pregnant mouse females often results in young with abnormally formed tails. Among the progeny of these abnormal mice, X-ray induced mutations may appear, but these are in no way more likely to affect the tail than any other part of the body.

Not only the frequency of abnormal children, but also that of miscarriages was increased among Japanese women who had been pregnant during exposure to the atom bomb: again, this was due to direct action of the radiation on the developing embryos, and frequency of abortion was normal among the rest of the surviving population. At first sight this is surprising to the geneticist. Embryonic death through dominant lethality is the most easily observed geneti-

cal effect after irradiation of male or female mice. It is highly probable that the atom bomb produced many dominant lethals in the human gametes which were exposed to it. Presumably, these lethals escaped detection because, like dominant lethals in the mouse, they killed the embryos at very early stages. Thus dominant lethality, although almost certainly a fairly frequent result of heavy irradiation, is not likely to cause human suffering or unhappiness. The same is true for translocations which, again by analogy with the mouse, are expected to be frequent among the children of heavily irradiated persons. The affected children will have drastically reduced fertility, and this condition will be transmitted as a dominant genetical change. In a species which habitually practises birth control, this is not likely to cause concern.

The kind of radiation damage which need cause concern is the production of harmful or lethal recessives which may be carried through a great many generations before they claim their victims. A recessive lethal will kill the first individual which received the mutated gene from both parents, or the first male which received it on his X-chromosome. A recessive harmful gene only increases the probability that those who carry it in homozygous condition will die early; eventually, however, it too will result in what has been called 'genetic death', and on the way to this end it will have produced a track of individuals who in some way, physically or mentally, are slightly inferior to what they would have been without this gene. In addition, it is likely that in man, as in *Drosophila*, many genes which are lethal or drastically harmful to the homozygote are slightly harmful to the heterozygote. Since many more persons inherit a harmful gene from one parent than from both, the slight effects on the heterozygotes will in their totality outweigh the much more drastic, but much rarer, effects on the homozygotes. Moreover, this slight deterioration will take effect even in the immediate progeny of the irradiated individuals.

Eventually, and at the cost of human frustration and suffering, the genetical effects of a single heavy dose of radiation will become eliminated from the population. This is not so for a population which is exposed to chronic low doses of irradiation. Because of the linear proportionality between radiation dose and mutation frequency, chronic irradiation at an approximately constant dose rate will, in effect, result in a mutation frequency which is permanently increased above its previous value. Under these conditions, harmful mutation will go on accumulating in the population until, after a great many generations, a new equilibrium is reached at which the rate of elimination of harmful genes through genetic death again equals the origin of these genes through mutation, and at which all harmful genes are present in higher frequencies than before. This applies to drastic hereditary de-

fects such as certain types of blindness or deafness or mental disease. It applies equally to the more intangible effects of genes which cause very slight disabilities of body or mind. Human variation is so great that it will hardly be possible to pinpoint those persons whose mental or physical abilities are slightly impaired by such genes; but over the population as a whole the deterioration will eventually become noticeable. Medical progress may to some extent mask it, but it cannot prevent it. Moreover, the picture of a human society in which most people are kept alive through constant medical supervision and treatment is not an attractive one.

The question is sometimes asked whether radiation can produce hereditary changes which have not occurred before and which may either cause new kinds of disease or may, through their very novelty, be a stimulus to further human evolution. The answer to this question is: 'No'. Ionizing radiation from natural sources has been producing mutations ever since the origin of life on earth, and all types of mutation which can be produced by its agency must already have occurred repeatedly.

Another question which is often raised is whether the benefit from useful mutations may not outweigh the damage done by the harmful ones. The answer is again: 'No'. In all investigated species harmful mutations are so overwhelmingly in excess of beneficial ones that planned genetical improvement through induced mutations is practicable only in species like grasses or bacteria in which one can afford to discard thousands of inferior progeny for the sake of a few superior ones. There is no reason to believe that the ratio of beneficial to harmful mutations will be different in the human species.

The control of genetical radiation damage.—The increasing use of ionizing radiation in medicine and industry makes it imperative to decide on permissible doses to which the individual and the population may be exposed without the risk that the harmful genetical effects may offset the benefit to be derived from X-rays, isotope therapy, nuclear power and other uses of radiation. Only a compromise solution is possible, for there is no lower threshold to the mutagenic action of radiation, and even if we were to abandon completely the use of ionizing radiation, cosmic radiation and naturally occurring radioactive substances would continue to produce genetical changes. We still lack data on which we could base an even moderately accurate estimate of the genetical effects of a given dose of radiation on human genes. Calculations use extrapolations from experiments on lower animals and include premises on which geneticists differ among themselves.

It was, therefore, surprising and encouraging that two independently prepared reports—one issued by the British Medical Research Council, the other by the

U.S.A. National Academy of Sciences—arrived at very similar limits for the estimated doubling dose, i.e. the dose which would double the present mutation rate. Both reports put the most probable limits at between 30 and 80 r received by the gonads from conception to the age of 30. In the final result, such a dose, if given generation after generation, would double the incidence of genetically caused defects, although for recessives this may take a very great number of generations. It has been calculated that in Great Britain there would be 200 more cases each of maniac depressive insanity and schizophrenia, and 1,500 more of severe mental deficiency among the first generation of 20 million births, and that similar increases would take place in each generation until the limiting value of twice the initial number was reached. For hereditary traits which show continuous variation about the normal, an increased mutation rate results in increased proportions of the extreme variants on either side of the mean; but if the trait has already been subjected to much selection towards one extreme, as is likely for human intelligence, it is mainly the minus variants which will be increased in number.

The maximum dose which the British report considers tolerable from a genetical point of view is 50 r to the gonads in excess of the natural radiation up to the age of thirty, and this should be received by not more than 1/50 of the total population. The American report recommends that records of the accumulated life-time exposure to radiation should be kept for every individual, that the average exposure of the population's reproductive cells to radiation above the natural background should be limited to 10 r from conception to age 30, and that individual persons should not receive a total accumulated gonad dose of more than 50 r up to age 30, and not more than 50 r additional up to age 40.

At present, by far the largest source of ionizing radiation impinging on human individuals is X-radiation used in medicine, especially in diagnosis. In Britain, X-ray diagnosis, mainly of hip, lumbar spine, lower abdomen and pelvis, adds at least 22% and possibly much more, to the average amount of natural radiation (3 r) which the gonads receive during the first 30 years of life. In the United States, this figure is considerably higher. H. J. MULLER, the discoverer of the mutagenic effects of X-rays, has been urging for many years that medical X-ray doses should not be higher than absolutely necessary, that wherever possible alternative methods of diagnosis and treatment should be used, and that in both patient and radiologist the reproductive organs should be shielded. The genetical dangers from the use of nuclear fission are at present negligibly small. It has been estimated that if nuclear weapon testing should continue at the present rate, this would raise the normal background radiation by only about 1%. Yet, since even the slightest increase

in the amount of background radiation will result in a proportionately increased incidence of harmful mutations, every precaution should be taken to keep all extra radiation from atomic energy processes as low as possible.

Mutagenic chemicals.—The search for mutagenic chemicals is almost as old as the modern science of genetics; but up to 1940 no clear proof for the production of genetical changes by any chemical had been obtained. During the last war this impasse was broken by the independent discovery of two mutagenic substances: mustard gas and urethane. These initial findings were soon followed by others, and at present the list of mutagenic substances is much too long for enumeration in a short article. It contains many chemicals which are related to mustard gas, and many carcinogens and anti-carcinogens. Of particular interest in the present context are three types of compound: (1) simple compounds like manganese chloride, hydrogen peroxide and formaldehyde, (2) alkaloids like morphine and atropine, and (3) purines like adenine, caffeine, theophylline. Some of these substances occur naturally in metabolism or are taken up by man in stimulants or medicines. It is too early to say whether, and in what concentrations or overall amounts, they can affect the chromosomes in human gametes. Most of them have so far proved mutagenic only in bacteria and flowering plants. Some have been found effective also in *Drosophila*. None have been tested on animals other than *Drosophila*. Yet in view of the rapid increase in the variety and amounts of chemical substances to which civilized man is exposed it is well to consider not only the harm which some of them may cause to individuals, but also the possibility that some of them may harm future generations by effects on human germ cells. Unfortunately, there is no cheap and easy way of testing for this possibility, for there is no reason to assume that chemicals, like X-rays, reach and attack the chromosomes in the germ cells of all organisms equally well. At the moment, small rodents are the only mammals which can be used in mutation work. Even this requires incomparably more money, space, time and effort than work on *Drosophila*; but the issue is important enough to justify this expenditure in money and scientific manpower.

Zusammenfassung

Die populären Auffassungen der durch Bestrahlung erzeugten genetischen Schädigungen irren gewöhnlich weit vom Ziele, und zwar in entgegengesetzten Richtungen. Oft hört man die Behauptung, dass bestrahlte Eltern die Gefahr laufen, Missgeburten oder sonstwie anormale Nachkommen zu haben. Noch öfter werden die genetischen Gefahren ionisierender Strahlen als unbeträchtlich und nicht beachtenswert hingestellt. In Wirklichkeit sind diese Gefahren sehr ernst zu nehmen. Sie betreffen aber nicht Individuen, sondern die menschliche Rasse als Ganzes. Über ihre Natur sind wir gut unterrichtet, über ihr Ausmass können wir uns

gegenwärtig nur sehr ungenaue Vorstellungen machen. Genetisch unerschwellige Strahlungsdosen gibt es nicht; die gesetzlich festgesetzte Toleranzdosis muss daher einen Kompromiss darstellen zwischen gegenwärtigem Nutzen und zukünftigen Gefahren.

¹ *The Hazards to Man of Nuclear and Applied Radiations*. Medical Research Council (London, H. M. Stationery Office, 1956).

² *The Biological Effects of Atomic Radiation: a Report to the Public from a Study by the National Academy of Sciences*. (National Academy of Sciences-National Research Council, Washington, D. C. 1956).

³ C. AUERBACH and A. ROBERTSON, *New Biology* 20, 30 (1956).

⁴ H. J. MULLER, *Bull. Atomic Scientists* 11, 329 (1955). (With many references to original papers.)

⁵ M. WESTERGAARD, *Impact of Science on Society* 6, 63 (1955).

Chemical Mutagenesis in Relation to the Concept of the Gene

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Attempts to enhance the mutation rates in plants and animals through chemical treatment go back to the pioneer work of MCDOUGAL (1911) in plants and to MORGAN's experiments with *Drosophila* in 1910. Although some earlier experiments gave suggestive results (especially the work of the Soviet scientists SACHAROV 1931–1936, LOBASOV 1934, and others¹), the real break-through came at the end of the Second World War in three different laboratories. In the Soviet Union, RAPOPORT (1946) demonstrated the mutagenic effect of formaldehyde on *Drosophila* and, in the years 1946–1948, this brilliant geneticist tested more than 20 chemicals for mutagenic effect on *Drosophila*, among others epoxides, dimethyl- and diethylsulphate, diazomethane, ethyleneimine, acrolein, and other unsaturated aldehydes, etc.² In Germany, OEHLKERS injected ethylurethane and potassium chloride into the flower buds of the plant *Oenothera*, whereby various types of chromosome mutations were induced³. These observations were extended to *Drosophila* by VOGT in Germany and by RAPOPORT. Simultaneously, in Scotland, AUERBACH, working in collaboration with the pharmacologist ROBSON, showed that a war gas, 'mustard gas', greatly enhances the mutation rate in *Drosophila*⁴.

Without exaggerating it may be said that these discoveries started an avalanche. To-day the list of mutagenic chemicals runs into many hundreds, ranging from inorganic salts to the most complex organic molecules⁵.

From the beginning, chemical mutagenesis was undoubtedly stimulated by its contact with cancer chemotherapy. Very soon 'mustard gas', and especially 'nitrogen mustard', was used in therapy against certain types of leukemia. In the last 10 years, many hundred chemical compounds have been tested as tumour inhibitors, and many of these new compounds were made available to geneticists to be tested for mutagenic activity⁶.

The fact that many mutagenic chemicals are completely unrelated with regard to chemical structure, physico-chemical and pharmacological properties, may give the impression that the whole field is in a rather chaotic state and that the study of chemical mutagenesis has been, to some extent, a disappointment with respect to the amount of fundamental biological and genetical information which has been obtained so far. The present author does not share this pessimism. Firstly, it should be remembered that this new branch of genetics is hardly more than 10 years old. Secondly, it seems possible even now to arrange the available data in such a way that they do not only stimulate further research but also provide new and important information about both fundamental and applied aspects of genetics. In order to draw such a pattern it is necessary, however, to keep two things in mind: (1) That the genetical mutation concept is very heterogeneous, ranging from point-mutations to polyploidy, and (2) that mutagens can act either directly on the gene, or indirectly by inhibiting antimutagens in the cells and thus interfering with the mutagen-antimutagen balance. The hydrogen peroxide-catalase-potassium cyanide system may be cited as an example of this type of interaction: H_2O_2 is mutagenic. Catalase is an antimutagen which destroys the mutagenic effect

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¹ H. STUBBE, *Handb. Vererb. Wiss.* II F (1938). — N. W. TIMOFEEFF-RESSOVSKY, *Biol. Rev. Cambridge* 9, 411 (1934).

² For references to RAPOPORT's publications (which are all in Russian) cf. I. H. HERSKOWITZ, *Amer. Nat.* 75, 181 (1951).

³ F. OEHLKERS, *Z. Ind. Abst.-Vererb.-Lehre* 81, 313 (1943).

⁴ CH. AUERBACH, *Nature* 157, 302 (1946).

⁵ Cf. CH. AUERBACH, *Biol. Rev. Cambridge* 24, 355 (1949). — E. BOYLAND, *Pharmac. Rev.* 6, 345 (1954).

⁶ O. G. FAHMY and MYRTLE FAHMY, *Proc. 5. Internat. Congr. Radiobiol.*, Stockholm 1956 (in press).

of H_2O_2 . If catalase is inhibited by KCN, the mutation rate is enhanced; not because KCN is mutagenic, but because the H_2O_2 concentration in the cells is increased⁷.

In order to prove the first point we must, however, try to integrate the mutation concept into the other basic genetical concepts. To this purpose it may be most convenient to define and describe the scope of genetics in terms of the modern language of cybernetics, information theory⁸. Genetics deals with the mechanism of transmission of genetic information from parent to offspring. The first problem—to what extent and in which way is the information which is expressed in the so-called phenotype of the parents transmitted to their offspring—has largely been solved: The genetical information (or rather information specificity) is transmitted from parent to offspring through self-reproducing particulate units, genes, which are organised in the chromosomes of the cell nuclei. The laws for transmission of information are expressed in the first and second Mendelian law and determined by the behaviour of the chromosomes during meiosis and fertilization.

After the solution of the first problem we are at once confronted with a second and most important one: How is the genetic information carried by the genes of the zygote translated to make its impact upon the offspring's phenotype?

It is well known that this is a very complicated process, since the phenotype is shaped through the interaction of two components, heredity and environment. The genetic information contained in the genes must be considered to be mere potentialities. What is actually decoded, and how it is decoded, depends upon the environment. Some of the more promising attempts at studying this interaction may perhaps be found in the work of BEALE and SONNEBORN on antigen differentiation in *Paramæcium*⁹.

It is fully realised that this somewhat unconventional approach to the fundamental genetical concepts may be too rigid and dogmatic. Still, it may be of more than just heuristic value to operate with the following three elements in genetical information theory:

(1) A self-reproducing information system, (2) a translation system which decodes the information and transmits it to (3) a receptor system.

It is likely that, if we accept these three elements, our system may be changed spontaneously or experimentally on three different levels: (1) in the receptor system or (2) in the translation system or (3) in the information system. Until now the starting point in all genetic analysis has been the phenotype, although

a direct chemical and even physical approach to the information system is within reach (due to the crystallographic analysis of the DNA molecule which led WATSON and CRICK to postulate a most suggestive double helix structure of DNA)¹⁰. Leaving this aspect aside for the moment, it may be stated that a given phenotypic variant may have arisen as a consequence of changes in either the receptor system, the translation system, or the information system. Fortunately, in most organisms the adequate tools are present for discriminating between these three events, namely the Mendelian crossing experiment, combined with microscopical, cytological observations.

It is again fully realised that it may be stretching a few points, when proposing to identify phenotypic variants due to changes in the receptor system with the so-called phenocopies. A phenocopy (GOLDSCHMIDT) is defined as an environmentally produced, non-inherited imitation of a known mutation¹¹. Such phenocopies can be induced by treating the organism with physical or chemical agents during certain sensitive stages of development.

It is also known that phenocopies can be induced rather specifically. By choosing the right chemicals and by proper timing of the treatment, almost 100% of the treated populations may sometimes show the same phenocopy. Also here RAPOPORT's studies on the induction of chemophænes are outstanding¹². This field of research may actually have been somewhat neglected by geneticists, and the possibilities for integrating the results of induction of phenocopies into the results of experimental mutagenesis are far from having been exhausted. It is important to keep in mind that phenocopies, which can be rather specifically induced, are not inherited, or, in other words, there is no feedback mechanism from the receptor system to the information system. However, we shall leave the phenocopies and turn to the next level of our three-phase system: Phenotypic variants due to alterations in the translation system.

In this field our present knowledge is possibly most deficient. However, we tentatively propose to identify such changes with so-called cytoplasmic mutations and use the important work of EPHRUSSI and his colleagues in Paris on the induction of the so-called 'petite' colonies in yeast as a model¹³. EPHRUSSI has shown that there are two kinds of 'petite' colonies in yeast, which both lack certain enzymes in the oxidative system. Some 'petites' arise as a result of nuclear mutations and the 'petite' character is then inherited according to a normal Mendelian scheme. Others which are due to cytoplasmic changes are

⁷ K. A. JENSEN, I. KIRK, G. KØLMARK, and M. WESTERGÅRD, Cold Spring Harbor Symp. Quant. Biol. 16, 245 (1951). —

⁸ N. WIENER, *Cybernetics* (New York, Paris 1955). — H. QUASTLER (Ed.), *Essays on the use of information theory in biology* (Urbana 1955).

⁹ G. H. BEALE, *The genetics of Paramæcium aurelia* (Cambridge 1954).

¹⁰ J. D. WATSON and F. H. C. CRICK, Cold Spring Harbor Symp. Quant. Biol. 18, 123 (1953).

¹¹ R. GOLDSCHMIDT, Z. Ind. Abst.-Vererb.-Lehre 69, 38 (1935).

¹² J. A. RAPOPORT, Amer. Nat. 81, 30 (1947).

¹³ B. EPHRUSSI, *Nucleo-cytoplasmic relations in micro-organisms* (Oxford 1953).

transmitted through the cytoplasm only. The latter, cytoplasmic 'petites' can be induced at a very high frequency by chemical treatment with acridines like euflavine. There is some evidence to believe that, in the cytoplasmic 'petites', something is wrong with the mitochondria of the yeast cells—and we know that the cytochrome-enzymes are located in the mitochondria. Hence, specific changes observed on the phenotypic level can, in this system, be pinned down with great precision to nuclear or cytoplasmic events, and the latter can be specifically induced by selecting the proper chemicals. Again there seems to be no feedback from the translation system to the information system. EPHRUSSI's work shows, in a beautiful and clear way, how a normal phenotype is produced only when both the information system (e.g. the proper nuclear genes) and the cytoplasmic component (which we here propose to identify with the translation system, ascribing an important role in the latter to the mitochondria) are functioning normally.

Let us now turn to the third level, where changes may result in a phenotypic variant: the information system, mutations proper. This is undoubtedly the category about which our knowledge is most satisfactory. For one thing, combined cytological and genetical studies have shown that all changes of this kind take place in the chromosomes, either in the individual genes (gene or point-mutations) or by changing the linear order or the number of the genes along the chromosomes (deficiencies, duplications, translocations or inversions). The latter types of mutations require at least one and mostly two breaks in the chromosomes. Finally, changes in the number of single chromosomes (aneuploidy) or chromosome sets (polyploidy) may also be responsible for changes in the information system which we classify as mutations. We shall, however, omit the two latter types from the following discussion.

Various genetical and cytological tests are available for the study of spontaneous and induced mutation rates, but it is important to know exactly which type of mutation is scored in each test.

(1) There are purely cytological tests for studying chromosome breakage. Plants with large and few chromosomes like *Allium*, *Vicia*, *Tradescantia*, *Oenothera* and others are mostly used. In the majority of cases, the genetical consequences of the chromosome breaks are not followed up in these tests.

(2) A method which is in a way complementary to the chromosome breakage test, is the so-called *Neurospora* back-mutation test. There are two main types of nuclear mutations (leaving aneuploidy and polyploidy out of consideration), namely (a) mutations which are due to one or two breaks of the chromosomes, and (b) changes which do not require, and do not seem to be associated with, breakages of the chromosomes: Gene- or point-mutations. It has always been difficult to

develop a clear-cut test for this latter important group, because the only way of deciding whether a mutation is due to point-mutation or chromosome breakage is by a process of elimination: If a mutation is *not* associated with visible changes in the chromosomes, if it is inherited in a regular Mendelian way, and if the crossing-over values are not distorted, we think we are dealing with a point-mutation. Actually, the existence of induced, at least X-ray induced, true point-mutations has sometimes been disputed, especially by STADLER¹⁴. The back-mutation test, where it is studied how a mutated gene reverts, through back-mutation, to the original 'wild type' allele, is probably as good a model for a point-mutation as may be expected to be found. We shall therefore consider the back-mutation test as a 'pure test' for point-mutations, complementary to the cytological tests for chromosome breakage.

(3) The classical methods for studying induced mutations, the *Drosophila* *ClB* test or its various modifications (the 'Muller-5' test) must be considered mixed tests, where the mutation yield is a mixture of mutations due to chromosome breakage and, possibly, point-mutations. However, it is also possible in *Drosophila*, by combined genetical and cytological methods, to score the rate of dominant lethals, and due to the ingenuity of Dr. MULLER and his students a number of stocks are now available in *Drosophila* which allow the separate study of various types of mutations (e.g. MULLER's 'multi-purpose' stock, and the 'maxy-stock'). In the latter, it is possible to score the mutation rate at 10–15 different, single *loci*. However, many flies are needed and much work has to be devoted to obtain quantitative information from such stocks which are only recently being used in the study of chemical mutagenesis.

(4) Very specific and interesting information has been obtained from the so-called 'prophage-activation test' in *Bacillus megatherium* developed by LWOFF and his colleagues at the Pasteur Institute¹⁵. Some bacteria are lysogenic. They carry a prophage which, when activated, is transformed into a phage which will lyse the bacteria which carry them. It is possible to increase the number of lysogenic cells in such strains (by activating the prophage) by means of radiation or by chemical treatment. It is very striking that the same chemicals which enhance prophage activation also induce back-mutations in *Neurospora*, whereas those which give negative results in the prophage-test are also inactive in the back-mutation test. However, since the yield of lysogenic particles may be 100% in such experiments, it is doubtful, whether we are here dealing with a true mutational event.

¹⁴ L. J. STADLER, *Science* 120, 811 (1954).

¹⁵ F. JACOB and E. WOLLMAN, Cold Spring Harbor Symp. Quant. Biol. 18, 101 (1953).

(5) In one of the classical objects in botanical genetics, *Antirrhinum*, OEHLKERS has developed a method for studying rates of visible mutations after treatment with chemicals. The mutation rates have been enhanced by compounds like urethane and various alkaloids, for instance morphine hydrochloride¹⁶.

(6) Finally, a great deal of work has been done on induction of mutations in bacteria, especially by using screening tests like resistance to penicillin, streptomycin or other antibiotics¹⁷. Unfortunately, it is not so easy to break down phenotypical changes in bacteria into the three possible categories, phenocopies, cytoplasmic mutations or nuclear mutations, as it is in higher organisms with a better understood sexual mechanism. We shall, however, omit the work on induction of bacterial mutations from the following discussions and confine ourselves mainly to the two complementary tests (1 and 2) for chromosome breakage and for point-mutations.

First a few words about the chemical induction of chromosome breakage. The pioneer work of OEHLKERS has been extended by many authors. In Sweden, LEVAN, TJIO, KIHLMAN, ÖSTERGREN, and others have shown that the chromosomes of *Allium* and *Vicia* can be broken by treatment with, for instance, phenols, quinones, benzpyrene, purines like 8-oxy-caffeine, by coumarin and others. D'AMATO in Italy has studied the effect of acridines, benzidine, acenaphtene and others. LOVELESS, REVELL, and FORD in England have investigated the effect of numerous alkylating agents, including many 'mustard' analogues, epoxides, ethyleneimine, triazine etc.¹⁸. The latter compounds have also been studied in *Drosophila*, especially by the FAHMY's at the Chester Beatty Research Institute in London¹⁹.

It is suggestive that chromosome breakage may result from a great variety of treatments. Breaks can be induced with a very high frequency by highly reactive, alkylating agents, but also by treatment with chemically very stable (but mostly very toxic) compounds which possibly act as enzyme poisons or which in some other way interfere with cell and chromosome metabolism. In this respect the induction of chromosome breakage has many characteristics in common with the induction of cancer. Also here one gets the impression that, if a cell is irritated long enough even by a very unspecific treatment, it may be transformed into a malignant cancer cell.

One great difficulty in dealing with the induction of chromosome breakage is the fact that parallel with the

process of induction of breaks goes the equally important problem of the future fate of the breaks: They may stay open, or they may reconstitute, e.g. the break may 'heal', or two different breaks may recombine in a new way. Probably most treatments which induce breaks also interfere with these processes. Very important recent work on these problems has been performed by SHELDON WOLFF at Oak Ridge²⁰. He has shown that there are two types of chromosome breaks, namely some which may stay open for 15 min or more, and which require energy for healing. If the ATP system of the cell is blocked by enzyme poisons like dinitrophenol, such breaks will remain open. The second type of breaks will stay open only 2-3 min, and they seem to reconstitute or recombine 'spontaneously', e.g. the healing process does not require energy. The first type of breaks is attributed to breaks in covalent bonds. The second type seems to be due to breaks in ionic bonds involving calcium or magnesium bonds. The possibility that the chromosome nucleoproteins are held together by ionic forces in calcium bonds was first suggested by MAZIA²¹. MAZIA, STEFFENSON, and SHELDON WOLFF have also shown that chelating agents, e.g. versenes (ethylenediamine tetraacetic acid) which remove calcium from the cells, induce chromosome breakage. The recent observation by TATUM and co-workers, that chelating agents also enhance the crossing-over frequency in the green alga *Chlamydomonas*, may have important consequences for our understanding of crossing-over as related to chromosome breakage²².

Finally, it should be mentioned that there is some evidence for a certain specificity in the chemical induction of chromosome breakage. Compounds like maleic hydrazide²³ and 8-oxy-caffeine²⁴ seem to induce breaks in the heterochromatin. However, this does not change the main impression conveyed by the work on chemical induction of chromosome breakage, namely that this is a fairly unspecific event; chromosome breakage can be induced by many different chemicals which have quite different physico-chemical and pharmacological properties, and chromosome breakage can probably be induced in various ways.

The results of the *Neurospora* back-mutation test provide a somewhat different picture. This test was worked out in 1946 by GILES at Yale University and, independently, by the present authors^{25,26}. It is well known that it is possible to induce so-called auxotro-

²⁰ S. WOLFF and H. E. LUIPPOLD, Proc. Nat. Acad. Sci. Wash. 42, 510 (1956).

²¹ D. MAZIA, Proc. Nat. Acad. Sci. Wash. 40, 521 (1954).

²² R. A. EVERSE and E. L. TATUM, Proc. Nat. Acad. Sci. Wash. 42, 68 (1956).

²³ J. MCLEISH, Heredity 6, Suppl. 107 (1953).

²⁴ B. KIHLMAN and A. LEVAN, Hereditas 37, 382 (1951).

²⁵ N. H. GILES, jr., Cold Spring Harbor Symp. Quant. Biol. 16, 283 (1951).

²⁶ K. A. JENSEN, I. KIRK, G. KØLMARK, and M. WESTERGÅRD, Cold Spring Harbor Symp. Quant. Biol. 16, 245 (1951).

¹⁶ E. OEHLKERS, Heredity 6, Suppl. 95 (1953).

¹⁷ M. DEMEREC, G. BERTANI, and J. FLINT, Amer. Nat. 85, 119 (1951).

¹⁸ A. LEVAN, Cold Spring Harbor Symp. Quant. Biol. 16, 233 (1951).

¹⁹ O. G. FAHMY and MYRTLE J. FAHMY, Heredity 6, Suppl. 149 (1953).

phic mutations in *Neurospora* and other microorganisms. Such mutations are unable to grow on the synthetic minimal medium which promotes the growth of the 'wild type' strains, because they lack a gene (or a gene has been inactivated) which in one way or another controls the activity of a specific enzyme that is essential for the synthesis of an indispensable growth factor, be it a vitamin, an amino acid, or a nucleic acid constituent. Normal growth of the mutant can be restored by adding the proper growth factor to the substrate. Some of these auxotrophic mutations are not stable, but revert with a low frequency to 'wild type', e.g. they are again able to grow on minimal medium without the additional growth factor. By conventional genetical methods, including analysis of linear tetrads, it is possible in *Neurospora* to identify the cause of a reversion, whether it is due to a non-genetic event (phenocopy), to a chromosome mutation, to a suppressor-mutation in a different *locus*, or to a true back-mutation²⁶. The back-mutation test is in principle a screening test where the non-mutated (non-reverted) cells are automatically eliminated on the minimal medium. It is therefore possible to test approximately 10^7 spores in a single experiment, and obtain reproducible dose-effect curves for various treatments.

The work from the present author's laboratory has mainly dealt with an adenine-requiring auxotrophic mutant which only reverts by back-mutation, whereas GILES started with a number of inositol-requiring mutants which by conventional genetical criteria would seem to be allelic, but which showed different spontaneous and radiation induced reverse mutation patterns. An important extension of this work is due to Dr. G. KØLMARK from our laboratory, who crossed one of GILES inositol-requiring strains with our adenine strain and isolated the double mutant. By using properly supplemented media, KØLMARK has been able to study the back-mutation pattern of the two mutations adenineless and inositolless on the same genetic and physiological background.

Some of the results of treating the single mutant (adenineless) with various chemical agents are shown in Table I. It will be seen that many chemicals are able to enhance the back-mutation frequency. However, although these chemicals are quite different in chemical structure, they have one trait in common: They are chemically very unstable and highly reactive compounds. Most are strongly alkylating agents, some may form free radicals or very reactive ions. In contrast to these positive results with the reactive compounds are our completely negative results with, e.g. phenols, 8-oxy-caffeine, urethane, maleic hydrazide, and others. All the latter chemicals can induce chromosome breakage, and in some cases also lethals or even visible mutations in *Drosophila* and in plants (*Antirrhinum*). They are chemically very stable and, although most

of them are toxic, all have failed to enhance the back-mutation rate in *Neurospora*.

It should be pointed out that the list of mutagenic chemicals given in Table I not only includes compounds which might be called abiological, in the sense that it is very unlikely that cells under natural conditions would be exposed to such chemicals, but also hydrogen peroxide and organic peroxides, which are certainly normal intermediates in cell metabolism. The mutagenic effect of hydrogen peroxide and organic peroxides was first discovered in bacteria by STONE and his collaborators in Texas²⁷, by DICKEY, CLELAND, and LOTZ²⁸, and by ourselves on *Neurospora*, and later extended to *Drosophila* (with respect to organic peroxides) by Mrs. ALTENBURG in USA²⁹ and by SOBELS in Utrecht³⁰. Its important perspectives are that these reagents link chemical mutagenesis with the study of X-ray and ultraviolet light induced mutations, since hydrogen peroxide and organic peroxides are certainly formed during the radio-chemical processes resulting from irradiation of cells. They also form a bridge between chemical mutagenesis and the problem of the origin of spontaneous mutations. It is known that only a very small fraction of the mutations in *Drosophila*, and perhaps not more than 10–15% in man, can be caused by 'natural' radiation, the rest must be due to chemical mutagens and probably also to thermal agitation. It is known, furthermore, that not only peroxides are formed during normal cell metabolism, but, as RAPOPORT has pointed out, even a strong mutagen like diethyl sulphate is sometimes likely to be formed during metabolism³¹.

The study of the mutagenic effect of peroxides has also been helpful in the understanding of one of the most important concepts in mutagenesis, the mutagen-antimutagen concept. If chemical mutagens play an important role in the origin of spontaneous mutations, there must obviously be a buffer system in the cells which counteracts such mutagens, in order to keep the spontaneous mutation rate as low as it is actually observed. At least one such natural antimutagen, namely catalase, is known. The mutagenic effect of hydrogen peroxide stops instantaneously if catalase is added, and in many organisms it has been impossible to induce mutations by hydrogen peroxide treatment, probably because the catalase content of the cells is too high. If this is true, we should expect enzyme poisons like potassium cyanide, which inactivates catalase, to be mutagenic, and this is also the case, as has been shown both in *Neurospora*²⁶ and more in-

²⁷ O. WYSS, J. B. CLARK, F. HAAS, and W. S. STONE, *J. Bact.* **56**, 51 (1948).

²⁸ F. H. DICKEY, G. H. CLELAND, and C. LOTZ, *Proc. Nat. Acad. Sci. Wash.* **35**, 581 (1949).

²⁹ L. S. ALTENBURG, *Proc. Nat. Acad. Sci. Wash.* **46**, 1037 (1954).

³⁰ F. H. SOBELS, *Nature* **177**, 979 (1956).

³¹ I. A. RAPOPORT, *Doklady Usesoyuz. Akad. Sci'sko Khoz. Nauk. im I. J. Lenina* **12**, 12 (1947).

The effect of various mutagens on the back-mutation rate of an adenineless strain (# 38701) of *Neurospora crassa*. The figures in the last column give the number of back-mutations per 10^6 treated macroconidia (the average number of nuclei in the macroconidia is 2-3) under optimal conditions. 'Optimal conditions' are defined as the treatment which induces the highest overall number of back-mutations, corresponding to the peak of the curves shown in Figure 1*.

Mutagen	Chemical formula	Mol. conc.	Time min	% survived	Mutations per 10 ⁶ conidia
1. Diepoxybutane	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}-\text{CH}-\text{CH}_2 \\ \diagup \quad \diagdown \\ \text{O} \quad \text{O} \end{array}$	0.2	40	56	85
2. Dimethyl sulphate	$(\text{CH}_3)_2\text{SO}_4$	0.005	30	44	64
3. Epichlorohydrin	$\text{H}_2\text{C}-\text{CH} \cdot \text{CH}_2 \cdot \text{Cl}$	0.15	45	42	56
4. Chloroethyl methane sulphonate (CB 1506)	$\begin{array}{c} \text{O} \\ \\ \text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{SO}_2 \cdot \text{CH}_3 \end{array}$	0.1	13	58	51
5. Glycidol	$\begin{array}{c} \text{H}_2\text{C}-\text{CH} \cdot \text{CH}_2 \cdot \text{OH} \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	0.5	60	26	34
6. <i>p</i> -N-di(β -chloroethyl)phenylalanine . CB 3025. <i>L</i> -form	$(\text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2)_2\text{N}-\text{C}_6\text{H}_4-\text{CH}_2-\text{CH} \cdot \text{NH}_2$ $\quad \quad \quad $ $\quad \quad \quad \text{COOH}$	0.03	40	100	22
7. Propylenoxide	$\begin{array}{c} \text{H}_2\text{C}-\text{CH} \cdot \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	0.5	60	27	21
8. Diethyl sulphate	$(\text{C}_2\text{H}_5)_2\text{SO}_4$	0.04	40	68	18
9. Ethyl methane sulphonate (CB 1528)	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{SO}_2 \cdot \text{CH}_3$	0.1	12.5	14	17
10. Ethylenoxide	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}_2 \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	0.025	15	63	17
11. Ethylenimine	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}_2 \\ \diagup \quad \diagdown \\ \text{NH} \end{array}$	0.05	30	75	16
12. Hydrogen peroxide + Formaldehyde	$\text{H}_2\text{O}_2 + \text{HCHO}$	0.06 + 0.3	30	20	4.3
13. 'Nitrogen Mustard' Di(β -chloroethyl) methylamine	$\text{H}_3\text{C} \cdot \text{N} \begin{array}{l} \diagup \text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl} \\ \diagdown \text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl, HCl} \end{array}$	0.0025	25	60	3.4
14. 1,2-monoepoxybutane	$\begin{array}{c} \text{H}_2\text{C}-\text{CH} \cdot \text{CH}_2 \cdot \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	0.2	40	47	3.2
15. <i>p</i> -N-di(β -chloroethyl)phenylanine CB 3026. <i>D</i> -form	$(\text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2)_2\text{N}-\text{C}_6\text{H}_4-\text{CH}_2-\text{CH} \cdot \text{NH}_2$ $\quad \quad \quad $ $\quad \quad \quad \text{COOH}$	0.03	120	90	3.1
16. Epibromohydrine	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}-\text{CH}_2\text{Br} \\ \diagup \quad \diagdown \\ \text{O} \end{array}$	0.08	45	40	2.5
17. Monochloro-'mustard' (β -chloroethyl) dimethylamine	$(\text{CH}_3)_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl, HCl}$	0.005	60	80	1.7
18. tert. butylhydroperoxide	$(\text{CH}_3)_3 \cdot \text{C}-\text{O}-\text{OH}$	0.09	30	50	1.5
19. Diepoxypropylether	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}-\text{CH}_2 \cdot \text{O} \cdot \text{CH}_2 \cdot \text{CH}-\text{CH}_2 \\ \diagup \quad \diagdown \quad \quad \quad \diagup \quad \diagdown \\ \text{O} \quad \quad \quad \text{O} \end{array}$	0.1	20	65	0.7
20. Triethylene-melamin (TEM)	$\begin{array}{c} \text{H}_2\text{C}-\text{CH}_2 \\ \diagup \quad \diagdown \\ \text{N} \\ \\ \text{C} \\ \diagup \quad \diagdown \\ \text{N} \quad \text{N} \\ \diagdown \quad \diagup \\ \text{N} \quad \text{C} \quad \text{N} \\ \diagup \quad \diagdown \quad \diagup \quad \diagdown \\ \text{H}_2\text{C} \quad \text{N} \quad \text{C} \quad \text{N} \quad \text{CH}_2 \\ \quad \quad \quad \quad \\ \text{H}_2\text{C} \quad \text{CH}_2 \quad \text{CH}_2 \end{array}$	0.02	50	55	0.6
21. Diazomethane	CH_2N_2	0.03	40	20	0.6
22. Trimethylphosphate	$(\text{CH}_3)_3\text{PO}_4$	0.2	40	96	0.5
23. Hydrogen peroxide	H_2O_2	0.2	45	10	0.4
24. Formaldehyde	HCHO	0.01	180	80	0.3

* Data compiled from K. A. JENSEN, G. KØLMARK, I. KIRK, and M. WESTERGÅRD, Cold Spring Harbor Symp. Quant. Biol. 16, 245 (1951). - G. KØLMARK and M. WESTERGÅRD, Hereditas 39, 209 (1953). - G. KØLMARK, Hereditas 39, 270 (1953). - G. KØLMARK and N. H. GILES, Genetics 40, 890 (1955). - G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. physiol. 28, 204 (1956); G. KØLMARK (unpublished).

directly in *Drosophila*, where treating the flies with KCN enhances the mutagenic effect of X-rays³². It seems likely that organic peroxidases in the cells have the same function as catalase and act as anti-mutagens. This discovery of the mutagen-antimutagen system has far reaching consequences for the understanding of the origin of spontaneous mutations as well as the action of mutagens. It is seen how chemicals can act not only directly, but also indirectly by destroying or inhibiting natural antimutagens in the cells. Hydrogen peroxide may be called a mutagen 'of the first order' whereas potassium cyanide is a mutagen 'of the second order'.

Some of KØLMARK's results with the double mutant are shown in Figure 1 and in Table II. These results

³² F. H. SOBELS, Z. Ind. Abst.-Vererb.-Lehre 86, 399 (1955).

add an interesting new pattern to the picture, namely the relative specificity of mutagens³³. As was shown in Table I, the adenine-mutant is very sensitive to treatment with, for instance, diepoxybutane. It was a great surprise to find that the inositol-mutant studied was almost resistant to this treatment. This does not mean, however, that the inositol-mutant always has a lower mutation rate. If ultraviolet light is used as a mutagen, the picture is reversed (Fig. 1a). However, most chemicals have induced very few mutations in the inositol strain with the remarkable exception of dimethyl sulphate and diethyl sulphate (Fig. 1b), the two compounds which were first tested

³³ G. KØLMARK, Hereditas 39, 270 (1955). — G. KØLMARK and N. H. GILES, jr., Genetics 40, 890 (1953). — G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 204 (1956).

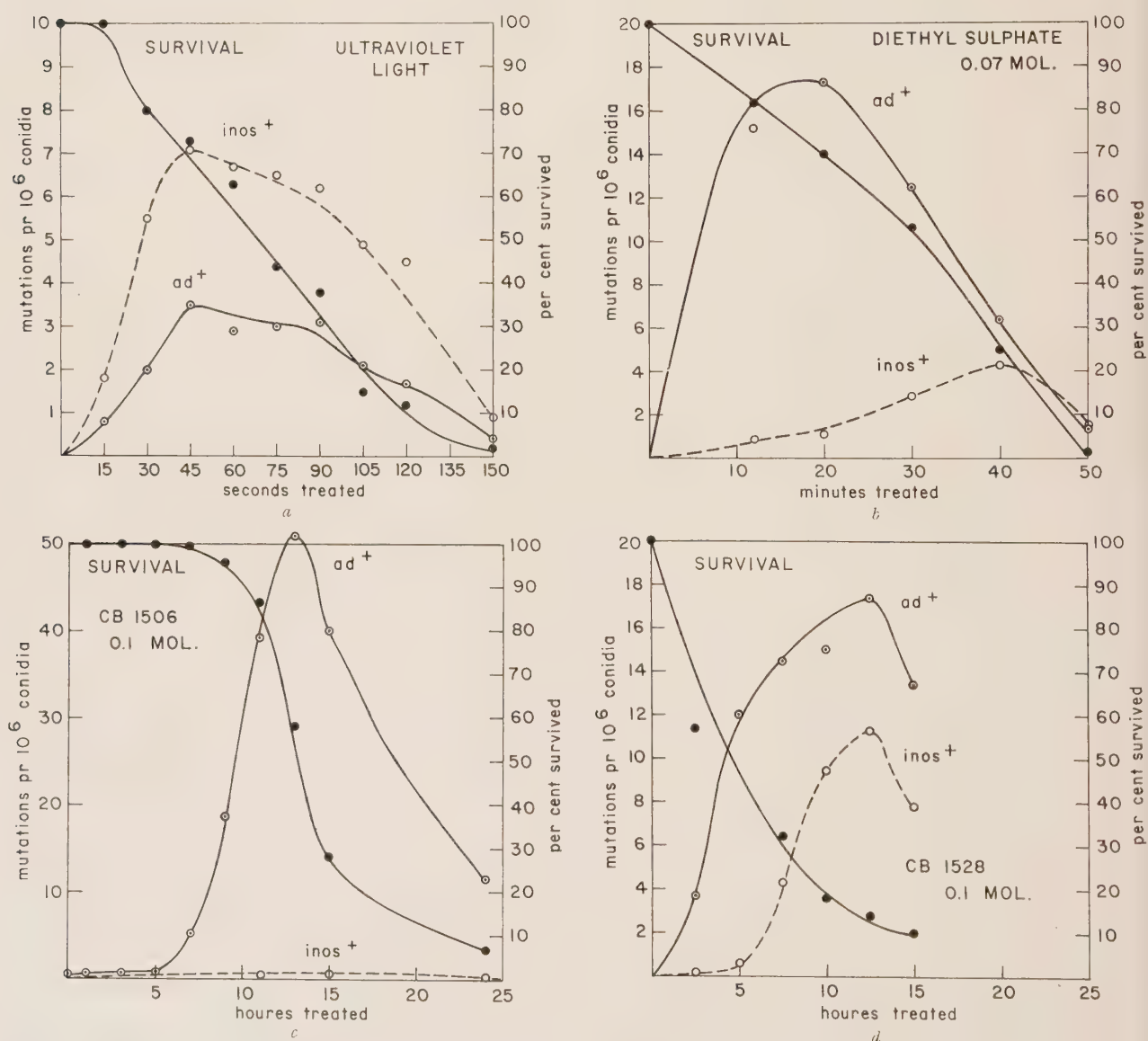


Fig. 1. The effect of 4 mutagens on the double mutant adenineless (# 38701), inositolless (# 37401). The right ordinate refers to the survival curve, the left ordinate to the number of back-mutations induced per 10⁶ treated conidia*. 1a: ultraviolet light; 1b: diethyl sulphate; 1c: chloroethyl methane sulphonate (CB. 1506, cf. table I for chemical formula); 1d: ethyl methane sulphonate (CB. 1528, cf. table I). Note that the scale of the left ordinate is not the same in all the graphs.

* Data from G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 204 (1956), and G. KØLMARK (unpublished).

Table II

The relative specificity of 6 mutagens on the double mutant inositolless (# 27401), adenineless (# 38701). The mutagens are compared under 'optimal conditions'*

	Mutations p.10 ⁶ conidia		Proportion ad ⁺ /inos ⁺
	inos ⁺	ad ⁺	
1. CB 1528**	11.3	17.4	1.5
2. Ultraviolet light	7.1	3.5	0.5
3. Diethylsulphate	4.3	16.8	4
4. Dimethylsulphate	3.4	64.0	19
5. CB 1506**	0.3	51.0	190
6. Diepoxybutane	0.2	89.0	445

* Data from G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 204 (1956); and G. KØLMARK (unpublished).

** See Table I for chemical structure.

on *Drosophila* by RAPOPORT³¹. More recently, KØLMARK (unpublished) has found an even more striking difference between two methane-sulphonate compounds, the chloroethyl and the ethyl derivatives (Fig. 1c and d). It is very interesting that the only two chemicals which appreciably enhance the reverse-mutation rate of the inositol mutant are both ethylating agents. Especially in the case of diethyl sulphate (Fig. 1b), it is obvious that the dosis-effect curves for the response of the two mutants are quite different; the maximum effect appears much later and at a much higher killing rate in the inositol mutant than in the adenine mutant. Reconstruction experiments have shown that selective killing cannot explain these differences³⁴. Table II summarizes this pattern of relative specificity of the two mutants after various treatments. It should be stressed, however, that these differences are not to be ascribed to the two *loci* controlling adenine and inositol synthesis, but they are characteristic of the two mutant alleles selected for these investigations, adenine strain # 38701, and inositol strain # 37401. Both GILES and KØLMARK have found other inositol mutants which behave in their reverse-mutation pattern like the adenine strain, and *vice versa*, and it should be kept in mind that many, and probably most, auxotrophic mutants are completely stable, or at least unable to revert through back-mutation. *The back-mutation pattern of a given mutant gene, as defined by its response to various physical and chemical mutagens, depends upon how the gene was originally damaged.*

However, the degree of destruction does not depend primarily upon the mutagen used. Recently, GILES and DE SERRES have shown that even X-ray induced mutations do not represent irreversible losses, because a number of X-ray induced adenine mutants are able to back-mutate, thus refuting the opinion, held by

STADLER, that all X-ray induced mutations are loss mutations³⁵.

Thus, it becomes clear, however, that the classical concept of the gene as a unit of mutation can no longer be maintained. The gene, defined as a functional unit, can be damaged in many different ways, there are many 'sites of damage' within the gene, and each mutation may be characterized by its specific reverse mutation pattern, spontaneous and induced. We shall revert to this most important point later. Two results should still be mentioned in order to complete this account of the back-mutation test.

KØLMARK and GILES³⁶ and KØLMARK³⁴ have been able to obtain further information about the mechanism of induction of back-mutations in the adenine *locus*. They have shown that the efficiency of a mutagen on the adenine strain is correlated with its dipole characteristics. By comparing the effect of a number of mono-functional epoxides, they find that the stronger the electro-positive charge of the carbonium ion, the stronger is the mutagenic effect. According to KØLMARK, the same is true for the alkyl esters³⁴. This suggests that the mutagen reacts with 'something' which carries an electro-negative charge in (or close to) the adenine gene. Such an ionic attraction may perhaps explain the 'relative specificity' of some chemical mutagens.

Finally, some important recent results obtained in GILES' laboratory should be mentioned. He has developed very precise methods for measuring the degree of phenotypic identity between the back-mutated strains and the 'wild-type' strain from which the mutants originated. The best results come from work on a gene which controls the last step in adenine synthesis, namely the splitting of adenylosuccinic acid into adenylic acid and fumaric acid³⁷. The enzyme responsible for this splitting (adenylosuccinase) has been isolated from 'wild-type' strains. However, mutants which are blocked in this terminal step lack this enzyme. In back-mutated, prototrophic strains which originated spontaneously or were induced by mutagenic treatment, the enzyme was again present. Quantitative studies of the enzyme activity showed, however, that the activity in the back-mutated strains was not up to the normal, e.g. 'wild-type' level. Does this mean that the back-mutations are incomplete, and that the original 'wild-type' gene has not been completely restored? This question can probably not be answered, but it might be pointed out that natural selection may come into the picture. This assumption is based upon the following line of reasoning: We start from a 'wild-type' gene which has long been exposed to, and presumably stabilized, through natural selec-

³⁵ N. H. GILES, jr., *Brookhaven Symp. in Biol.* 8, 103 (1955).

³⁶ G. KØLMARK and N. H. GILES, jr., *Genetics* 40, 890 (1955).

³⁷ N. H. GILES, jr., *Intern. Genetics Symp. Japan* 91, (1956) and personal communication.

³⁴ G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 204 (1956).

tion. This gene is inactivated and probably partly destroyed by a mutagenic treatment. By further mutagenic treatment, a back-mutation is induced. We may perhaps consider the back-mutated gene as a 'new gene', not yet 'lubricated' and stabilized through natural selection. It will be very interesting to learn whether the enzymatic activity in Dr. GILES' back-mutated strains can be restored to normal level through natural and artificial selection.

A certain optimism with respect to chemical mutagenesis as an important tool for both theoretical and applied genetics was expressed at the beginning of this paper. The reason for this optimism may be summarized in the following paragraphs.

(1) There is no overall theory which can explain the induction of all types of mutations. Each type of mutations (point-mutations, mutations due to chromosome breakage, cytoplasmic mutations, etc.) must be dealt with separately. It is therefore necessary to have specific quantitative methods for studying the different mutational events separately. Such methods are now available.

(2) It has been shown that chromosomes can be broken by many different chemicals which act through various mechanisms (alkylating agents, enzyme poisons, chelating agents, etc.). Point-mutations (using back-mutations as a model), on the other hand, are induced only by a limited group of chemicals which are very unstable and reactive. The release of free energy through the reaction of such compounds with cell constituents may be a decisive factor in the ultimate mutational event. Probably all mutagens which induce point-mutations also break chromosomes, but not all 'chromosome mutagens' are able to induce gene (back) mutations.

(3) A further important step towards the understanding of the action of chemical mutagens comes from the knowledge that mutagens are counterbalanced by antimutagens. The mutation rate may therefore be enhanced by chemicals which act either directly (mutagens of the first order) or indirectly, by destroying or inhibiting natural antimutagens (mutagens of the second order). The hydrogen peroxide-catalase-potassium cyanide system may, as already mentioned, serve as a model for such a mechanism.

(4) A number of chemical mutagens, such as hydrogen peroxide and organic peroxides, and probably others, must be considered 'natural mutagens' and are responsible for at least part of the spontaneous mutations in plants, animals, and man. They therefore deserve the same attention as 'natural radiation' when problems such as the origin of spontaneous mutations are discussed.

(5) We now begin to realize that there is no sharp demarcation line between the physical and chemical approach to the study of mutations; the gap is bridged by the discovery of the mutagenic effect of peroxides

and organic peroxides. The recent discovery by SOBELS of the synergistic effect of formaldehyde or potassium cyanide and X-ray treatment on the mutation rate in *Drosophila*, and SHELDON WOLFF's demonstration of a similar effect of versene treatment and X-rays on *Vicia*, should be mentioned in this connection.

(6) We are—and should be—much concerned about genetical radiation hazards, because man-made radiation (from the widespread use of X-rays in medical therapy and diagnosis, and from peaceful and military uses of atomic energy) has increased the natural background radiation by perhaps 25%. However, perhaps not more than 10% of the spontaneous mutations in man are due to natural radiation. It is, in the present author's opinion, quite possible that man's internal as well as external chemical environment has been changed even more drastically by 'man-made chemicals': Internally through the growing use of stimulants, drugs, and antibiotics. Even weed-killers may be concentrated in the human body *via* the vegetables we eat. It is known that most antibiotics and many drugs are mutagenic, because they will break chromosomes in *Vicia*, *Allium*, etc. Maleic hydrazide, which has been widely used in agricultural practice, is also a powerful mutagen. Even more important is perhaps the change in our external chemical environment. Exhaust fumes from automobiles and factories are creating the notorious 'smog' over many big cities, and it is known that organic peroxides are major constituents of this 'smog'—and that peroxides are mutagenic. We may soon have to take as drastic steps to protect our genes from man-made chemical mutagens as we take in trying to protect our descendants from man-made radiation.

However, because of the close (although not yet fully understood) relation between mutagenesis and carcinogenesis, these problems concern our own generation too. Many chemical mutagens are also carcinogenic (although the correlation may not be 100%). Fortunately, there is a more optimistic aspect to the mutation-cancer problem, because some chemical mutagens are also tumour inhibitors and used as such in cancer chemotherapy.

(7) The most important aspect of chemical mutagenesis as a tool in fundamental genetical research is perhaps the new possibility for characterizing individual mutant alleles. The development of adequate screening tests has made it possible to study quantitatively mutation rates, and especially back-mutation rates, of single genes, and the individual mutant allele can now be characterized not only by its spontaneous mutation frequency and by its response to X-rays and ultra-violet light, but also by its response to many chemical mutagens. As we have seen, the consequence of this improvement of our analytical tool has been *that the concept of the gene as a unit of function and of mutation has broken down.*

It should be pointed out that this situation is completely parallel to the one which now exists with respect to the reevaluation of our concept of the gene as a unit of recombination. Screening methods (again mainly in microorganisms) have been developed by which it is possible to test for crossing-over events of the order of 10^{-8} . Thereby the phenomenon of intra-allelic crossing-over has been discovered. (In *Aspergillus* by PONTECORVO and his group in Glasgow³⁸; in *Neurospora* by GILES²⁵, MITCHELL³⁹, and others; in bacteria by DEMEREC⁴⁰; in virus by SEYMOUR BENZER⁴¹.) Recombination within the gene (between what, from a functional point of view, must be considered alleles of the same *locus*) is also a fact in *Drosophila*⁴². In the classical 'white-series' of multiple alleles, 'white' and 'eosin' are now for the first time on the same chromosome. Whether it will be necessary to introduce the new concept of 'Gene Conversion' to explain some phenomena connected with intra-allelic, meiotic interaction (as suggested by LINDEGREEN) remains to be seen (for further discussion see LINDEGREEN⁴³, MITCHELL³⁹, EMERSON⁴⁴, PRITCHARD³⁸, SANSOME⁴⁵, and WINGE⁴⁶).

In conclusion a few general comments on the impact of these results upon our concept of the gene: Some geneticists think that they have completely invalidated the old concept, but this is not necessarily true. At least to the present author, the situation is rather clear. From the beginning, genetics operated with three gene-concepts: The gene as a unit of function, the gene as a unit of recombination, and the gene as a unit of mutation⁴⁷. Until 10–15 years ago, everybody was satisfied, because the gene as a functional unit was at the same time the recombinational and mutational unit. In recent years, the resolving power of our genetical analytical tools for studying recombination as well as mutation has been increased by a factor of 1000 or more. It is possible to test millions of gametes in much less time than it took before to test hundreds or thousands. This has given much more detailed information about the fine-structure of the gene⁴⁸. How-

ever, the tools for studying mutational and recombinational events are now so refined that in using them we interfere more and more with the functional integrity of the gene. As a consequence, the two approaches to the study of the gene, the functional and the mutation-recombination approach, are gradually becoming mutually exclusive. This does not mean, however, that the new data on subgenic mutations and recombinations are inconsistent with the concept of the functional integrity of the gene; but it is not unlikely that we are approaching a situation in biology which has for years been known in physics, namely the principle of complementarity⁴⁹. The results of different approaches to the study of the gene should not be considered mutually exclusive, but complementary.

There is, however, one aspect of present day genetical research which seems somewhat distressing. Classical genetics was a synthesis of results from genetical crossing experiments and visible light microscopy. Today the resolving power of the genetical tools is pushed far beyond that of light microscopy, which is practically useless in the study of phenomena such as intra-allelic crossing-over, point-mutations etc. The electron microscope may have sufficient resolving power, but it has been unable to serve its purpose in genetics because of many technical difficulties with fixation and dehydration. In the meantime, new tools such as cytochemistry, crystallography, and the use of isotopes, provide unforeseen possibilities for studying genetical mechanisms on a molecular level. The WATSON-CRICK model of the DNA-molecule, with its possibilities for explaining DNA replication, has already been mentioned. It is, however, still an open question how far we dare generalise from this model. It is true that the brilliant isotope experiments of HERSHEY and others have shown that, in bacterial viruses, the genetical information must be in the DNA⁵⁰. However, we also know that in plant viruses like TMV (Tobacco Mosaic Virus) the genetical information is probably in the RNA⁵¹; in higher organisms with organized chromosomes it may well be the nucleoproteins which are the carriers of genetical information. Despite the many brilliant and stimulating papers on the genetical function and replication of DNA as visualized from the WATSON-CRICK model⁵², the next major breakthrough in genetics may come when the cytological approach can again keep pace with genetical analysis; when the electron microscope takes its proper place in genetical analysis, when we can study the submicro-

³⁸ R. H. PRITCHARD, *Heredity* 9, 343 (1955).

³⁹ MARY B. MITCHELL, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 285 (1956).

⁴⁰ M. DEMEREC *et al.*, *Carnegie Publ.* 612 1 (1956).

⁴¹ S. BENZER, *Proc. Nat. Acad. Sci. Wash.* 41, 344 (1955).

⁴² E. B. LEWIS, *Amer. Nat.* 89, 73 (1955). — M. E. MCKENDRICK and C. PONTECORVO, *Exper.* 8, 309 (1955). — Cf. also the classical 'lozenge' case in *Drosophila*: M. M. GREEN, *Proc. Nat. Acad. Sci. Wash.* 35, 586 (1949).

⁴³ C. C. LINDEGREEN, *Science* 121, 605 (1955).

⁴⁴ S. EMERSON, C. r. Trav. Lab. Carlsberg, Sér. Physiol. 26, 71 (1956).

⁴⁵ EVA SANSOME, C. r. Trav. Lab. CARLSBERG, Sér. Physiol. 26, 315 (1956).

⁴⁶ Ø. WINGE, *Heredity* 9, 373 (1955).

⁴⁷ S. BENZER, in 'The Chemical Basis of Heredity' (Baltimore 1957).

⁴⁸ It should be pointed out that already in 1940, MULLER discussed this problem of the divisibility of the genetic material. See H. J. MULLER and D. RAFFEL, *Genetics* 25, 541 (1940).

⁴⁹ N. BOHR, *The Unity of knowledge* (Doubleday, New York 1955), p. 47. — M. DELBRÜCK, *Trans. Connecticut Acad. Arts. Sci.* 38, 173 (1949).

⁵⁰ Cf. A. D. HERSHEY and M. CHASE, *J. Gen. Physiol.* 36, 39 (1952).

⁵¹ H. FRAENKEL-CONRAT and R. S. WILLIAMS, *Proc. Nat. Acad. Sci. Wash.* 41, 690 (1955).

⁵² M. DELBRÜCK and G. STENT in 'The Chemical Basis of Heredity' (Baltimore 1957).

scopic basis for processes like intra-allelic crossing-over, forward- and back-mutations, 'gene conversion' etc. This day may not be far off.

Acknowledgement.—The author is much indebted to Dr. GUNNAR KÖLMARK for permission to quote many of his unpublished data. He also wants to thank Dr. CHARLOTTE AUERBACH, Edinburgh, Professor H. J. MULLER, Bloomington, and Dr. K. G. ZIMMER, Stockholm, who have read the manuscript and, without sharing any responsibilities for its content, have offered much stimulating criticism.

Zusammenfassung

Die vorliegende Arbeit gibt einen Überblick über neuere Untersuchungen chemischer Mutationsauslösungen mit besonderer Betonung der Induktion von Chromosomenbrüchen in Pflanzen und Rückmutationen bei *Neurospora*. Die Arbeit zeigt, dass jedes Allel ein charakteristisches Mutationsverhalten zeigt, das durch die Reaktion auf physikalische und chemische Mutagen definiert wird. Diese Ergebnisse werden unter dem Aspekt diskutiert, dass ein Gen die Einheit der Funktion, Rekombination und Mutation darstellt.

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. — Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. — Per le brevi comunicazioni è responsabile solo l'autore. — The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Der Einfluss der «Lorentz-Kontraktion» der Erde auf den Gang der Quarzuhren. II

Aus Vergleichen von Quarzuhren mit Pendeluhr, die am Observatorium in Neuenburg angestellt wurden, ergab sich, dass die Standunterschiede zwischen den zwei Uhrentypen innerhalb von nur etwa $\pm 0,01$ s einen linearen Verlauf nehmen, ihre Gangschwankungen also nahe gleich sein müssen. Da es nun möglich ist, für eine Pendeluhr eine theoretische Gangformel aufzustellen, welche die Wirkung der «Lorentz-Kontraktion» auf die Schwingungsdauer des Pendels ausdrückt, so kann also dieselbe Formel auch bei Quarzuhren Anwendung finden.

In meiner Abhandlung «Die beobachtete Gangschwankung der Quarzuhren und die «Lorentz-Kontraktion» der Erde»¹ habe ich die von mir abgeleitete Formel angegeben. Sie enthält in ihrem weit überragenden täglichen bzw. jährlichen Hauptglied den Faktor $\sin 2\varphi$, wo φ die Polhöhe der Beobachtungsstation bezeichnet. Daraus folgt, dass die von der «Lorentz-Kontraktion» der Erde hervorgerufene Gangschwankung in südlichen Breiten das umgekehrte Vorzeichen haben müsste wie in nördlichen, während natürlich eine etwaige Jahresschwankung in der Rotationsdauer der Erde überall gleich herauskommen würde. Die Jahreskurven der relativen Uhrkorrekturen von Quarzuhren an nördlichen und südlichen Stationen, bezogen auf das Mittel der Uhrkorrekturen an allen Stationen, werden sich daher spiegelbildlich verhalten.

Dass dies vollkommen zutrifft, habe ich schon in einer früheren Arbeit mit dem gleichen Titel² gezeigt, auf die ich hier bezüglich aller Einzelheiten verweise. Es konnten dort die an den Stationen Greenwich, Potsdam, Buenos Aires und Mount Stromlo erhaltenen Jahreskurven für 1948, 1949, 1950 wiedergegeben werden.

¹ L. COURVOISIER, Astronom. Nachrichten 281, 259 (1954).

² L. COURVOISIER, Exper. 9, 286 (1953).

Inzwischen liegen nun aber auch die im «Bulletin Horaire du Bureau International de l'Heure» (Paris) verzeichneten relativen Uhrkorrekturen für 1951, 1952, 1953, 1954 vor, und sie sollen hier in entsprechenden graphischen Darstellungen als Fortsetzung der schon publizierten Jahreskurven folgen. Für die nördlichen Kurven wurde das jeweilige Mittel N der in Greenwich und Potsdam beobachteten relativen Uhrkorrekturen benutzt, für die südlichen das Mittel S der Beobachtungen an den zwei Stationen in Buenos Aires (Geodätisches Institut und Naval Observatorium). Die nachstehende Tabelle enthält die Monatsmittel dieser relativen Uhrkorrekturen, die dann in den vier Figuren für jedes Jahr graphisch ausgeglichen sind.

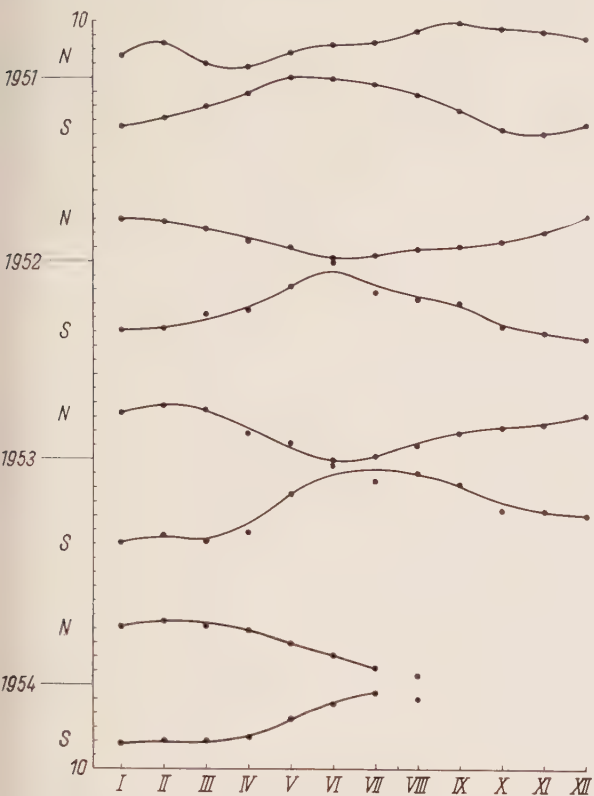
Wie man sieht, kann gar kein Zweifel darüber bestehen, dass auch bei diesen neuen Jahreskurven der relativen Uhrkorrekturen von Quarzuhren die an den südlichen Stationen erhaltenen Kurven, abgesehen von der natur-

Monatsmittel der relativen Uhrkorrekturen (0,001 s)

$$\left(N = \frac{G + P}{2}, \quad S = \frac{B_{Ag} + B_{An}}{2} \right)$$

Monat (Mitte)	1951		1952		1953		1954	
	N	S	N	S	N	S	N	S
Januar	+ 5	-14	+20	-10	+22	-28	+21	-22
Februar	+16	- 8	+18	- 8	+28	-23	+26	-21
März	0	0	+12	+ 3	+26	-28	+23	-20
April	- 2	+ 8	+ 6	+ 6	+ 8	-22	+19	-18
Mai	+ 7	+21	0	+22	+ 2	+ 6	+ 9	- 4
Juni	+14	+19	- 8	+40	-11	+27	0	+ 7
Juli	+15	+16	- 6	+18	- 8	+15	- 8	+15
August	+23	+ 7	- 2	+14	0	+20	-13	+10
September . .	+29	- 3	0	+10	+ 9	+12		
Oktober	+24	-17	+ 4	- 6	+12	- 6		
November . . .	+23	-21	+10	-12	+16	- 5		
Dezember . . .	+18	-13	+22	-15	+22	-10		

gemässen Veränderlichkeit der Form der Kurven selbst, ganz allgemein Spiegelbilder der in der Nordhemisphäre beobachteten Jahreskurven sind. Es dürfte wohl kaum



einen deutlicheren Beweis für die Existenz der «Lorentz-Kontraktion» geben.

L. COURVOISIER †

Riehen bei Basel (11. Februar 1957), im Oktober 1955.

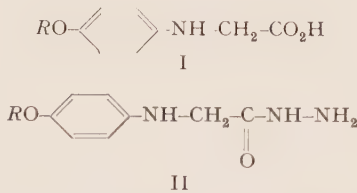
Summary

In addition to his paper², which contains the yearly curves 1948, 1949, 1950 of relative corrections of Quartz-clocks on northern and southern stations, the author gives here the four new curves for 1951, 1952, 1953, 1954 with the results of the observations in Greenwich, Potsdam and Buenos Aires (geod. and naval.). The theoretical statement that the curves at southern stations must agree with the reflexes of those on northern stations has now still better foundations than before and thus the existence of the «Lorentz-contraction» is still more clearly proved.

Sur l'activité tuberculostatique des N-arylglycines para-substituées

Récemment, BERSCH et DÖPP¹ ont montré que certaines N-arylglycines para-substituées possèdent une activité tuberculostatique considérable vis-à-vis de leur souche de bacilles tuberculeux; ainsi, la N-(p-éthoxyphényl)-glycine (I; R = C₂H₅) montre une action in-

hibitrice aux concentrations de 1/200 000^e en milieu de Dubos, et à des concentrations inférieures à 10⁻⁶ en milieu de Sauton, et la N-(p-n-butoxyphényl)glycine (I; R = n-C₄H₉) accuse



une activité tuberculostatique encore plus élevée.

Afin d'étudier les relations entre activité tuberculostatique et structure moléculaire dans cette série, nous avons examiné le pouvoir inhibiteur d'une série de N-arylglycines nouvelles ou déjà connues sur la croissance de *Mycobacterium tuberculosis var. hominis* (souche H 37 RVD). Le milieu utilisé est celui de Dubos (au tween 80 et à la fraction albuminique V de COHEN), l'inoculum correspondant à 0,01 mg de bacilles en poids humide pour 5 cm³ de milieu de culture; l'inhibition de la croissance est mesurée par opacimétrie à l'aide de l'électrophotomètre de Dognon, la durée d'incubation étant de 12 jours. Les résultats sont consignés dans le tableau suivant:

Substance	Activité à 10 ⁻⁵
N-p-anisylglycine	—
N-o-éthoxyphénylglycine	—
N-p-éthoxyphénylglycine	—
N-(4-n-propoxyphényl)glycine	+
N-(4-n-butoxyphényl)glycine	+
N-(4-n-amyloxyphényl)glycine	+
N-(4-isoamyloxyphényl)glycine	+
N-(4-n-heptyloxyphényl)glycine	+
N-(4-éthylphényl)glycine	—
N-(4-n-propylphényl)glycine	—
N-(4-fluorophényl)glycine	—
N-(4-chlorophényl)glycine	—

En ce qui concerne les relations entre structure chimique et pouvoir tuberculostatique, l'influence favorable des radicaux alkyloxy supérieurs est analogue à ce qu'on avait déjà constaté dans le cas des thiocarbanilides 4,4'-disubstitués². D'autre part, la conversion des N-arylglycines en hydrazides correspondants (II) détruit l'activité tuberculostatique à la concentration de 10⁻⁵; c'est aussi le cas pour les 1-acyl-4-arylthiosemicarbazides préparées en faisant agir des isothiocyanates d'aryles sur les hydrazides (II).

Au cours d'expérience *in vivo*, les N-(p-alkyloxyphényl)glycines ont montré une toxicité tellement prononcée qu'elles ne semblent pas devoir présenter d'intérêt thérapeutique.

N. P. BUU-HOI, N. D. XUONG,
N. B. TIEN, J. M. GAZAVE,
J. PILLOT et M^{lle} G. DUFRAISSE

Institut du Radium de l'Université de Paris, le 4 janvier 1957.

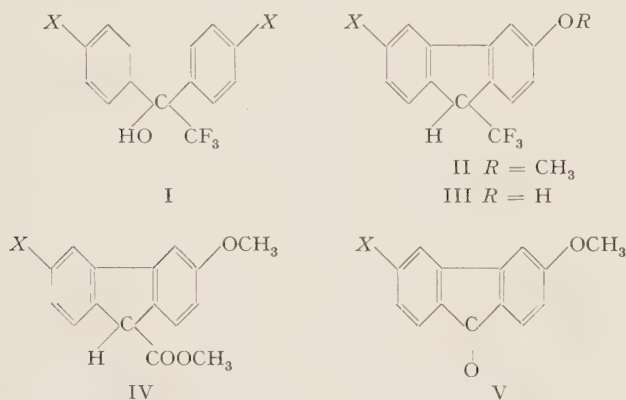
¹ H. W. BERSCH et W. DÖPP, *Arzneimittelforschung* 5, 183, 335 (1955).
² R. L. MAYER, P. C. EISMAN et E. A. KONOPKA, *Proc. Soc. exp. Biol. Med.* 82, 769 (1953). — N. P. BUU-HOI et N. D. XUONG, *C. r. Acad. Sci.* 237, 498 (1953).

Summary

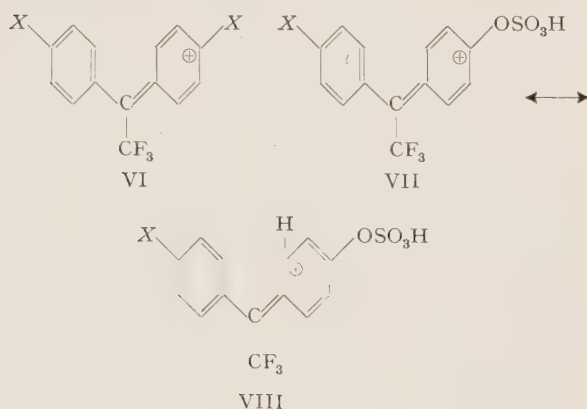
Several compounds belonging to the group of *para*-substituted N-arylglycines have been found to exhibit pronounced tuberculostatic activity *in vitro*, but are too toxic for practical application. The relationship between chemical structure and activity is similar to that observed in the group of thiocarbanilides.

A Cycloisomerization Reaction of Di-(*p*-halogenophenyl)-trifluoromethyl-carbinols

Di-(*p*-halogenophenyl)-trifluoromethyl-carbinols (I, $X = \text{halogen}$)¹ dissolve in concentrated sulphuric acid with an intensely purple colour ($\lambda_{\text{max}} = 570\text{--}580 \text{ m}\mu$) which changes rapidly to orange ($\lambda_{\text{max}} = 495 \text{ m}\mu$); this change is accompanied by formation of the corresponding hydrohalogenic acid, HX. When the orange solution is poured into water, III ($X = \text{halogen}$, $R = \text{H}$; m.p. $192\text{--}193^\circ$) is formed. When methyl alcohol is employed instead of water, the methyl ether II is obtained. In a similar cyclodehydration reaction benzoic acid is converted by aluminium chloride into fluorene-9-carboxylic acid².



The structure of II was proven as follows: the trifluoromethyl group is converted easily by methanolic alkali to a carbomethoxy group (IV; m.p. $129\text{--}130^\circ$). Oxidation of IV with alkaline hydrogen peroxide gave a yellow ketone



¹ E. D. BERGMANN, A. S. TAHORI, A. KALUSZYNER, and S. REUTER, *Nature* 176, 266 (1955). – A. KALUSZYNER, S. REUTER, and E. D. BERGMANN, *J. Amer. chem. Soc.* 77, 4146 (1955).

² D. VORLAENDER, *Ber. dtsch. chem. Ges.* 44, 2467 (1911).

$\text{C}_{14}\text{H}_9\text{ClO}_2$ (m.p. $181\text{--}182^\circ$), which was shown to be 3-methoxy-6-chlorofluorenone (V) by an unambiguous synthesis, starting from 4-chloro-anthranilic acid and anisole.

The following mechanism for the formation of II from I ($X = \text{Cl}$) appears reasonable. In the carbonium ion VI which is formed when I is dissolved in concentrated sulphuric acid, one of the halogen atoms is non-aromatic and reacts with the acid to $\text{VII} \leftrightarrow \text{VIII}$. This is cyclized with elimination of a proton; the product reacts with methanol or water to give II or III, respectively.

S. COHEN and A. KALUSZYNER

Research Laboratories, Medical Corps, Israel Defence Forces, January 22, 1957.

Résumé

Une solution de di-(*p*-chlorophényl)-trifluorométhyl-carbinol dans l'acide sulfurique concentré, diluée à l'eau ou au méthanol, donne naissance à du chloro-3-hydroxy- (ou méthoxy-) -6-trifluorométhyl-9 fluorène. Le composé méthoxylé, après l'alkoolyse alcaline du groupement $-\text{CF}_3$, a été dégradé au fluorénone correspondant, dont la synthèse a été réalisée indépendamment.

Quantitative Determination of Bone Minerals from Roentgenograms

Densitometric measurements of radiograms have been much used to estimate the mineral content of bones¹. However, the apparatus required is expensive and complicated and the technique is tedious. Moreover, because the materials which absorb roentgen rays are not randomly distributed, subjective judgement plays an important role in the selection of the areas to be measured. Besides the danger of subjective bias, the uneven distribution of minerals renders the interpretation of the data obtained by density measurements rather difficult, as has been shown in connection with histological sections².

For obviating the distribution error, ORNSTEIN³ described a new photographic method. A specimen to be examined was photographed. The emulsion was developed to produce a γ value = 1. A positive print was then made using a photographic enlarger. The positive image was developed to produce a γ value = 1. The images of individual nuclei were cut out of the film, and the amount of silver per image was determined. ORNSTEIN stated that the use of silver estimation method in film blackening measurements might be at least as accurate as the densitometric scanning methods. Moreover, it is faster and requires fairly simple and inexpensive apparatus.

Theoretically the same method could be expected to apply equally well to quantitative roentgenological studies of bones⁴. ORNSTEIN's method has been modified

¹ W. Mc FARLAND, *Science* 119, 810 (1954).

² D. GLICK, A. ENGSTRÖM, and B. G. MALMSTRÖM, *Science* 114, 253 (1951). – O. ERÄNKÖ and J. KIHLEBERG, *Quantitative methods in histology and microscopy histochemistry* (S. Karger, Basel and New York; Little, Brown & Co., Boston and Toronto 1955).

³ L. ORNSTEIN, *J. Lab. Invest.* 1, 250 (1952).

⁴ I am grateful to Prof. O. ERÄNKÖ from the Department of Anatomy, University of Helsinki, for suggesting the use of silver analysis.

by me and the results hitherto obtained with the modified method seem promising.

Basal phalanges of human fingers were found convenient for this kind of study. The hand was radiographed in a perspex tray with a layer of water, as JACKSON⁵ has proposed. JACKSON's method was modified by using only a 3.5 cm thick layer of water and placing a 1–2 cm thick plate made of a modified Columbian paste under the basal phalanges to be examined. The thickness of the plate used depended on the thickness of the basal phalanx to be examined, because the distance between the upper surface of the finger and the bottom of the perspex tray was kept constant. The modified Columbian paste contained following agents:

Paraffin wax	90.0 g
Beeswax	110.0 g
Birch saw dust	28.0 g

This paste corresponded to the absorption qualities of the soft parts surrounding finger phalanges.

The roentgenograms were developed to produce a γ value around 3. Two reference systems were used for

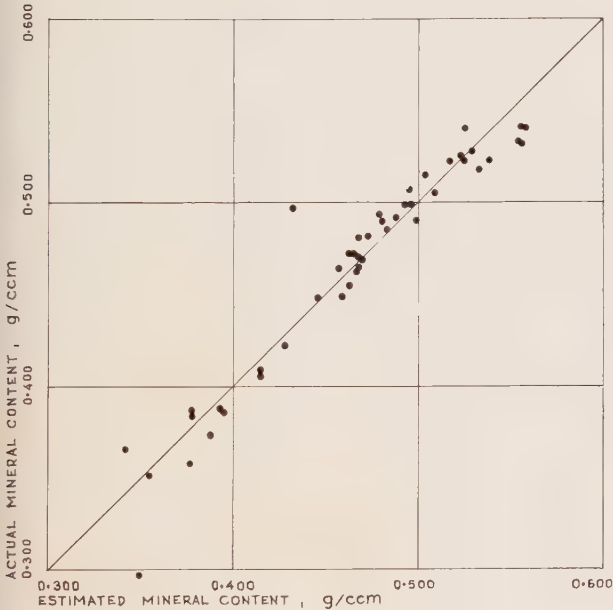


Fig. 1.

the density control of films. The other consisted of perspex boxes containing various amounts of bone powder made of beef bones. The other reference system was a set of standard bones, i.e. basal phalanges with varying mineral contents of fingers obtained from autopsied subjects. These basal phalanges were embedded into a layer of the modified Columbian paste corresponding to the soft parts and a 1–2 cm thick layer of the modified Columbian paste was placed under them to make their distances to the bottom of the perspex tray constant. Both reference systems were usually radiographed together with the hand to be examined. The tube distance was fixed at 1.6 m, and the exposure was 40 mAs at 60 kVp with a standard X-ray machine,

Siemens Roentgenkugel. The films were developed with continuous agitation for 5 min at 20°C with Kodak's D-19b developer. The fixation, washing and drying were made under strictly standardized conditions. Kodirex non-screen roentgen film was used. The images of the phalanges to be examined and those of the standard bones and the perspex boxes were cut out of the radiograms. The areas of these images were then measured and the silver contents determined. Instead of the various methods mentioned by MEES⁶, VOLHARD's indirect titration⁷ was used taking into account the common principles of accuracy for volumetric methods stated by CONWAY⁸. This determination proved to be fast and inexpensive and the error of method proved to be moderate, within the range used about 0.3%.

To check the accuracy of this roentgenological method, left hands of 86 autopsy subjects were radiographed as described. Every hand was exposed twice on two pieces of Kodirex non-screen film placed on another. Thus, four roentgenograms were obtained in every case. Curve was prepared in which the silver content was plotted against the thickness of bone powder layer in the perspex boxes. Only such films, from which a good curve was obtained and the silver contents of the images of the standard bones were within previously determined limits, were used in analyses. These conditions were fulfilled in 46 cases. In these cases the basal phalanges were removed and their volume and mineral contents were determined. The Figure shows the estimated mineral content as plotted against the actual content found. The error of method of such an estimate was calculated by regression analysis (ERÄNKÖ *et al.*²) to be about 3.6%, and the statistical possibility to obtain an error greater than about 7.2% is 5 times in 100 analyses⁹.

This method has also been used in a study on the mineral contents of the phalanges of the fingers in living subjects suffering from rheumatoid arthritis (to be published).

P. VIRTAMA

Department of Anatomy, University of Helsinki, December 5, 1956.

Zusammenfassung

Eine neue röntgenologische Methode für die Bestimmung des Mineralgehaltes im Knochen wird beschrieben. Die Genauigkeit konnte durch Vermeidung der Fehler, die wegen der unregelmässigen Verteilung des absorbierenden Materials entstehen, erhöht werden.

⁶ C. E. K. MEES, *The theory of the photographic process* (The Macmillan Company, New York 1954).
⁷ A. J. VOGEL, *A text-book of quantitative inorganic analysis. Theory and practice* (Green and Co., London, New York, and Toronto 1953).
⁸ E. J. CONWAY, *Microdiffusion analysis and volumetric error* (Crosby Lockwood & Son, London 1950).
⁹ Mr. J. KIHLEBERG has calculated by multiregression analysis the optimum equation necessary for obtaining the estimates. I am very grateful to him.

⁵ H. JACKSON, Brit. J. Radiol. 24, 613 (1951).

Pre- and Postreaction pH of two Specifically Interacting Large-Molecule Systems*

LANDSTEINER¹ believed that the attraction between oppositely charged groups, such as COO^- and NH_3^+ , contributed importantly to the formation of the antibody-antigen bond. If a number of such groups form part of each specific combining site, as suggested by LANDSTEINER and later authors², it might well be thought that some net liberation or absorption of hydrogen ions would accompany serological reactions, a possibility not entirely ruled out by the experiments of SMITH and MARRACK³, for the antibody and antigen solutions used by them were not, by modern standards, very concentrated.

SINGER and CAMPBELL⁴ however, have suggested that in some antibody-antigen systems, at least, only a single ionized carboxyl group (in antibody or antigen) with presumably a complementary NH_3^+ in the corresponding site of the other reagent, is involved in each bond. Since we believe antibody to be bivalent, this would mean the liberation or absorption of at the most 2 moles of H^+ per mole of antibody, and unless the pK of the NH_3^+ is considerably changed by the combination, probably much less. Furthermore, the demonstration that the specific portions of the blood group substances⁵ do not contain any ionized groups seems to show that some antibody-antigen bonds do not involve the mutual attraction of COO^- and NH_3^+ at all.

In view of the divergence between these more recent views and the conclusions to which LANDSTEINER¹ was led by his classical work, we felt it worth while to re-examine the question with use of modern concentrated reagents and modern equipment. The negative results are presented briefly here as setting an upper limit to the amount of H^+ liberated or absorbed in two typical reactions involving antigens.

Experimental.—As a classical antibody-antigen system we selected horse antitoxin and diphtheria toxoid. The concentrated antitoxin contained 4200 standard units per ml, nearly 4 times the strength of the preparation available to SMITH and MARRACK³. It contained 6.48 mg N per ml. The toxoid, containing 4200 Lf units per ml, was reported by the Massachusetts Public Health Biologic Laboratories, which supplied it, to be 100% pure. It contained 1.85 mg N per ml.

In order to obtain data from another system as different as possible, and at the same time to take advantage of other available concentrated reagents, we used blood group A substance and the plant agglutinin (lectin) from lima beans⁶. Our lectin preparation contained 2.06 mg N per ml, of which about 30% was

specifically precipitable. The A substance was prepared from commercial hog gastric mucin by MORGAN's method⁷; one third of it was specifically precipitable. Since nothing derived from serum was involved in the lectin-A substance reaction, it is not strictly a serological system. The close similarity in behavior of lectins and antibodies⁸, however justify its study here.

Before the reagents were mixed, they were adjusted to the same pH (studies were carried out over a range from pH 4.00 to 7.50) by the addition of the requisite amount of 0.05 N HCl or NaOH. The reagents were mixed in optimal proportions⁸ at room temperature in amounts to give about 25 ml. The pH was determined, with the pH meter of the Cambridge Instrument Co., immediately after mixing, 15 min later, and after 24 h in the refrigerator. Six successive readings were made at each time. In the case of the antitoxin and toxoid, strict asepsis was preserved although control experiments indicated this was not necessary.

No detectable pH changes followed mixing the reagents at any pH tested.

In order to determine the limiting sensitivity of the experiments, mixtures of the reagents were titrated with 0.1 N HCl and NaOH, and the buffer capacity of the mixture computed at the pH where it was a minimum (pH 7 for the first system and 7.5 for the second). From the buffer capacity of the system and the observed variations in the pH readings, it could be determined, with use of FISHER's test⁹ for the significance of the difference between two means, how small a release or absorption of hydrogen ions could have been detected. For the antitoxin-toxoid system this was 2.22 equivalents of H^+ per mole of antitoxin, and for the lectin-A substance system 0.95 Eq. H^+ per mole of lectin.

It would seem that our experiments are a further indication that the pH changes, if any, accompanying these large-molecule reactions are slight, in line with recent work. They probably do not exclude the possibility that if antigens containing large numbers of ionized groups were used, a pH change could be observed.

We are grateful to Dr. H. E. BOWEN of the Massachusetts Public Health Biologic Laboratories for generous gifts of antitoxin and toxoid, and to Dr. E. A. KABAT for a sample of purified A substance.

W. C. BOYD and B. BASKYS

Boston University, School of Medicine, Boston, Mass.,
February 5, 1957.

Zusammenfassung

Diphtherietoxoide und Antitoxin einerseits, gruppenspezifisches Pflanzenagglutinin und Blutgruppensubstanz andererseits, wurden auf verschiedenes pH gebracht und das erste und das zweite Paar gemischt, ohne dass eine pH-Änderung eintrat. Es wird eine Erklärung versucht.

* The work reported in this paper was made possible by support extended to Boston University by the National Science Foundation (G-1583) and by research grants (H-1076[C4] and RG 4104 M & G) from the National Heart Institute, National Institutes of Health, Public Health Service.

¹ K. LANDSTEINER, *The Specificity of Serological Reactions*, 2nd Ed. (Harvard University Press, 1945).

² W. C. BOYD, *Fundamentals of Immunology*, 3rd Ed. (Interscience Publishers, 1956).

³ F. C. SMITH and J. MARRACK, *Brit. J. exp. Path.* 11, 494 (1930).

⁴ S. J. SINGER and D. H. CAMPBELL, *J. Amer. chem. Soc.* 77, 3504 (1955).

⁵ E. A. KABAT, *Blood Group Substances. Their Chemistry and Immunochemistry* (Academic Press, 1956).

⁶ W. C. BOYD, E. SHAPLEIGH, and M. McMASTER, *Arch. Biochem. and Biophys.* 55, 226 (1955).

⁷ D. AMINOFF, W. T. J. MORGAN, and W. M. WATKINS, *Biochem. J.* 46, 426 (1950).

⁸ H. R. DEAN and R. A. WEBB, *J. Path. Bact.* 29, 473 (1926).

⁹ R. A. FISHER, *Statistical Methods for Research Workers*, 11th Ed. (Oliver and Boyd, Edinburgh, 1950).

Über die Verbesserung der Eiweisshydrolyse durch Proteinvorbehandlung mit Perameisensäure

Nach SCHRAM *et al.* besteht die Möglichkeit, Zystin und Zystein als Zysteinsäure nach Perameisensäurevorbehandlung und anschliessender HCl-Hydrolyse der Proteinträger quantitativ zu bestimmen¹. Zysteinsäure wurde als relativ hydrolysestabil befunden. TOENNIES und HOMILLER² nehmen an, dass Perameisensäure in gewissem Umfang mit einer Reihe von Aminosäuren schon im Eiweissverband reagiert.

Die vorliegenden Untersuchungen wurden an Fischmehl vorgenommen, um quantitativ die Ausmasse eventuell zu erwartender Umwandlungsprozesse nach erfolgter Behandlung mit Perameisensäure und anschliessender HCl-Hydrolyse zu ermitteln. In einer vorausgegangenen Untersuchung³ waren am gleichen Fischmehl Aminosäureanalysen nach MOORE und STEIN vorgenommen worden, um einen Überblick über den chemischen Ablauf der HCl-Hydrolyse nach DUSTIN⁴ in gestaffelten Zeitabständen zu gewinnen. Die Daten dieser Versuche sollen in vorliegender Arbeit zum Vergleich herangezogen werden.

Nach den Angaben von HIRS⁵ war mit der Umwandlung von Zystin bzw. Zystein, Methionin und Tyrosin zu rechnen. SANGER⁶ erhielt bei ähnlichen Versuchen neben Tyrosin ein Derivat, das er als Tyrosin X bezeichnete. HIRS⁵ identifizierte dieses Artefakt als Chlorotyrosin, das stets dann nach Perameisensäurevorbehandlung auftrat, wenn das zu oxydierende Substrat mit Cl⁻ verunreinigt war. Im Rahmen von Futtermitteluntersuchungen wird es nur sehr schwer sein, die vorhandenen Proteine verlustlos chlorfrei zu gewinnen. Es wurde versucht, das Tyrosin quantitativ in Chlorotyrosin zu überführen.

Als geeignetes Oxydationsreagens erwies sich ein Gemisch von 0,5 ml 30%igem H₂O₂ (MERCK, Darmstadt) und 4,5 ml 98%iger Ameisensäure (MERCK, Darmstadt) bei 50°C. 3 min nach der Mischung der beiden Komponenten erfolgte die Zugabe von je 0,8 ml der Perameisensäure zu je 20 mg Protein, dem 20 mg NaCl beigemischt worden waren. Die Reaktion wurde bei 50°C im Wasserbad durchgeführt und nach 15 min durch Verdünnen mit 200 ml H₂O je 20 mg Protein unterbrochen. Unmittelbar anschliessend konnte die Flüssigkeit im Vakuum bei etwa 40°C bis zur Sirupkonsistenz des Rückstandes abdestilliert werden. Der so gewonnene Rest wurde dann der HCl-Hydrolyse nach DUSTIN⁴ 24 h lang unterworfen.

Aus dem Vergleich der Aminosäurewerte der normalen 24-, 48- und 72-h-Hydrolyse der Tabelle mit den Daten des 24-h-Hydrolysates des mit Perameisensäure vorbehandelten Fischmehls geht hervor, dass bei sämtlichen Aminosäuren des oxydierten Fischmehls Maximalwerte vorliegen. Es scheint durch den Einfluss der Ameisensäure einerseits eine gewisse Schutzwirkung auf hydrolyselabile Aminosäuren⁷, zum anderen eine Katalyse bei

	Aminosäuregehalt in % nach normaler Hydrolyse			Perameisensäure- Vorbehandlung und 24-h-HCl- Hydrolyse
	24 h	48 h	72 h	
Asparaginsäure . . .	7,25	7,52	7,65	7,65
Threonin	3,72	3,56	3,48	3,82
Serin	3,56	3,38	2,87	4,33
Glutaminsäure . . .	11,35	10,17	9,27	11,38
Glycin	5,80	6,12	5,42	6,12
Alanin	6,89	5,61	5,56	6,90
Valin	6,59	4,75	4,36	6,63
Methionin	1,99	1,87	1,18	2,19
Isoleuzin	1,34	2,75	3,74	3,75
Leuzin	4,12	5,12	6,53	6,65
Tyrosin	2,31	2,21	2,14	2,31
Phenylalanin . . .	1,93	2,20	2,27	2,46
Histidin	1,56	1,04	1,05	1,62
Lysin	4,79	4,33	4,04	4,81
Arginin	0,62	0,59	0,58	0,62
Zystein 1/2				0,79

der Spaltung der gegen kochende HCl relativ resistenten Peptide³ zu erfolgen.

Die säulenchromatographische Bestimmung der entstehenden Eiweisspaltprodukte nach HCl-Hydrolyse und vorangehender Perameisensäurevorbehandlung erfolgt am besten nach der von HIRS⁵ modifizierten Methode nach MOORE und STEIN⁸. Zystin und Zystein erscheinen auf dem Eluierungsbild als Zysteinsäure, Methionin als Methioninsulfon und Tyrosin als Chlorotyrosin. Die Bestimmung der Zysteinsäure ist nach dem genannten Verfahren dann möglich, wenn keine anderen sehr sauren Substanzen im Analysenmaterial vorliegen. Dies kann durch ein Probechromatogramm eines normalen HCl-Hydrolysates bzw. auf papierchromatographischem Wege ermittelt werden. Zysteinsäure kann bei gleichzeitiger Anwesenheit sehr saurer, ninhydrin-positiver Substanzen nach SCHRAM¹ an Dowex-2-Säulen chromatographisch bestimmt werden. Chlorotyrosin wird auf einer 15-cm-Amberlite-IR-120 (XE-69)-Kolonne vollständig vom Phenylalanin getrennt. Im Eluierungsbild eines Chromatogrammes an Dowex-50-X4-Säulen erscheint Chlorotyrosin unmittelbar neben Histidin, zum Teil wird Histidin überlappt. An der Position des Tyrosins im Chromatogramm der 100-cm-Dowex-50-X12-Kolonne konnte ebenfalls keine ninhydrin-positive Reaktion festgestellt werden. Demnach ist unter den beschriebenen Versuchsbedingungen sämtliches Tyrosin in Chlorotyrosin übergegangen.

Weitere Untersuchungen zur Frage der Schutzwirkung bzw. Katalyse bei der HCl-Hydrolyse durch Vorbehandlung der Substrate durch Perameisensäure sind im Gange.

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Universität Bonn, den 18. Januar 1957.

Summary

By treatment of fish-meal with performic acid, it is possible to convert methionine, cystine and tyrosine residues in the intact protein into products stable to hydrolysis. After hydrolysing with hydrochloric acid, maximum values of amino acids were found, compared with amino acid values of non-treated 24, 48 and 72 h hydrolysates. It is assumed that unstable amino acids are protected and the cleaving of relative HCl-resistant peptides is accelerated by performic acid.

⁸ S. MOORE und W. H. STEIN, J. biol. Chem. 211, 893 (1954)

¹ E. SCHRAM, S. MOORE und E. BIGWOOD, Biochem. J. 57, 33 (1954).
² G. TOENNIES und R. P. HOMILLER, J. Amer. chem. Soc. 64, 3054 (1942).
³ G. KRAMPITZ, Naturwissenschaften (im Druck).
⁴ J. P. DUSTIN, C. CZAJKOWSKA und S. MOORE, Analyt. chim. Acta N. Y. 9, 256 (1953).
⁵ C. H. W. HIRS, J. biol. Chem. 219, 611 (1956).
⁶ F. SANGER und H. TUPPY, Biochem. J. 49, 463, 481 (1951).
⁷ S. U. GURNANI, U. S. KUMTA und M. B. SAHASRABUDHE, Biochem. biophys. Acta 16, 553 (1955).

Action du rayonnement X sur la croissance des cellules internodales de l'algue *Chara vulgaris* L.

Dans un précédent article¹, nous avons montré que chez *Hordeum sativum* Jess. il était possible de reproduire avec des rayons X mous, un phénomène mis en évidence par SCHWARTZ² chez le maïs avec les neutrons et les rayons γ du Co^{60} . Ce phénomène a été réétudié par cet auteur en 1956³. Il y a absence de proportionnalité entre certaines doses de rayons X et leur effet biologique quand, après irradiation des graines sèches, on mesure les croissances des plantules issues de ces graines. Ainsi, les hauteurs des coléoptiles diminuent lorsque la dose augmente de 50 kr à 200 kr; elles s'accroissent ensuite lorsqu'on augmente la dose de 200 kr à 400 kr, puis diminuent légèrement au-dessus de cette dose.

Chez le maïs, SCHWARTZ² a proposé l'explication suivante du phénomène: quand la dose augmente, les graines sont tuées, aucune division cellulaire ne pouvant plus avoir lieu. La croissance accrue par des doses supérieures serait due à une inactivation de certains systèmes enzymatiques. Quand ces activités enzymatiques sont affectées, les plantules atteignent le maximum de hauteur exclusivement par élongation cellulaire.

Qu'advierait-il si on irradie des tissus dont la croissance s'effectue normalement toujours par élongation à l'exclusion de divisions cellulaires? Pour répondre à cette question, nous avons choisi comme matériel d'expérience les grandes cellules internodales de *Chara vulgaris* L. qui atteignent 2 à 4 cm de longueur. Elles sont polynucléées et les noyaux sont répartis dans toute la masse cytoplasmique. Jamais, nous n'avons observé de phénomène mitotique dans ces cellules. Nous avons pensé qu'un tel matériel conviendrait parfaitement à nos investigations.

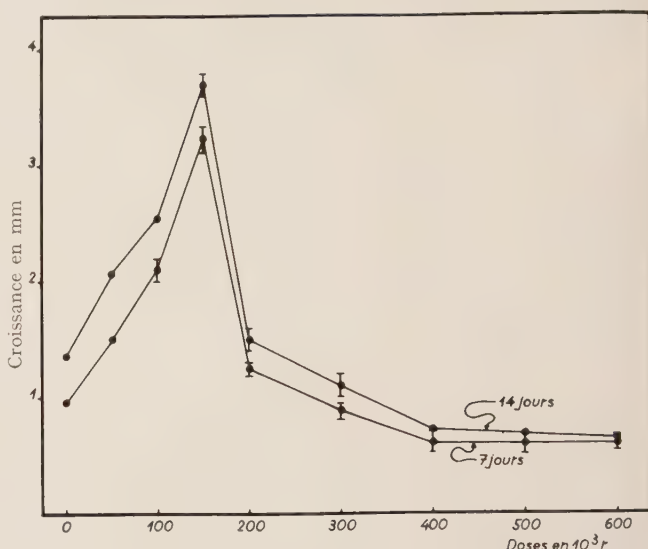
Conditions expérimentales. Les grandes cellules internodales de *Chara* sont sectionnées de part et d'autre de chaque groupe de cellules nodales. Les «feuilles» disposées en verticilles aux nœuds sont sectionnées à la base. Nous isolons donc ainsi un groupe formé de grandes cellules internodales et, aux extrémités, deux groupes de cellules nodales, c'est-à-dire un entre-nœud.

Pour chaque dose de radiations, nous préparons 20 entre-nœuds dont les tailles fluctuent entre 30 et 35 mm. Il s'agit de cellules adultes.

Pendant la période d'irradiation, l'entre-nœud repose sur du papier buvard humecté d'eau de la distribution. Les conditions d'irradiation sont les suivantes: tube à fenêtre de beryllium fonctionnant sans filtre, 40 kV, 25 mA, distance focale 12 cm, champ de 5 cm de diamètre, débit environ 9 kr/min; doses de 50 kr à 600 kr⁴.

Après irradiation, les entre-nœuds sont transférés en boîte de Petri de 10 cm de diamètre contenant 50 cm³ d'eau d'étang. Pendant la durée de l'expérience, la température oscille entre 21 et 22°C, l'éclairement est de 2000 Lux pendant 16 h sur 24. Les mesures de cellules sont réalisées au micromètre oculaire 4, 7, 14 et 21 jours après la fin de l'irradiation.

Résultats. Quatre jours après le début du traitement, les élongations sont encore trop peu appréciables et les accroissements cellulaires n'ont pas été portés sur le graphique.



Croissance de l'entre-nœud isolé de *Chara* après irradiation aux rayons X (40 kV). En abscisse, doses en kr. En ordonnées, croissance réelle (en mm) de lots de 20 entre-nœuds mesurée 7 ou 14 jours après irradiation. Le départ des courbes indique la croissance des témoins. Le σ est figuré sur les courbes.

La Figure montre que, après 7 jours, la croissance cellulaire augmente au fur et à mesure que la dose augmente en allant de 0 à 150 kr, qu'elle diminue fortement au delà de 150 kr jusqu'à 400 kr. Au-dessus de cette dose, la croissance reste approximativement la même; les cellules ont un aspect nécrotique; elles se décolorent. Au-dessus de la dose de 300 kr, la croissance est moindre pour les cellules irradiées que pour le témoin.

Après 14 jours, la courbe possède la même allure qu'après 7 jours et les points se confondent pratiquement depuis la dose de 200 kr jusqu'à 600 kr. Entre 14 et 21 jours, il n'y a plus donc pratiquement de croissance.

L'interprétation de cette expérience est simple puisqu'aucune mitose n'existe dans les cellules internodales de *Chara*. On enregistre, à l'état pur, l'action du rayonnement X sur l'élongation. L'accroissement d'élongation observé chez *Chara* semble correspondre à l'augmentation de croissance enregistrée chez l'orge pour des doses comprises entre 200 et 400 kr.

Nous pensons que, sous l'action de doses accrues de rayons X, il y a libération de facteurs de croissance, mais l'origine et la nature de ces facteurs restent à préciser.

J. MOUTSCHEN

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Summary

We have irradiated internodal cells of *Chara vulgaris* L. at different doses of X rays from 50 to 600 kr. These internodal cells never show mitotic activity and are of plasmodial constitution. When elongations are measured 7, 14 and 21 days after irradiation, we observe increased elongations from 0 to 150 kr. Then, elongations diminish when doses increase from 150 to 600 kr. This absence of proportionality between doses and biological effect is believed to be in relation to the increased amount of certain enzymes liberated by X rays.

¹ J. MOUTSCHEN, Z. M. BACQ et A. HERVE, Exper. 12, 314 (1956).

² D. SCHWARTZ, Science 119, 45 (1954).

³ D. SCHWARTZ, The American Naturalist 90, 323 (1956).

⁴ Nous remercions le Docteur A. HERVE qui a bien voulu procéder à ces irradiations au Laboratoire de Radiothérapie de l'Université de Liège.

On the Isolation and Cultivation of Intracellular Symbiotes of *Oryzaephilus surinamensis* L. (Cucujidae: Coleoptera)

Association of intracellular bacterium-like micro-organisms with *Oryzaephilus surinamensis* L. was first discovered and studied by PIERANTONI¹. Later KOCH² worked out their morphology, development and mode of transmission from one generation to the succeeding one. He proved that they are passed on to the progeny by means of ovariole infection in the early stages of development of egg in the female beetle. PANT and FRAENKEL³ extended the studies to include the functional

The sterilizing fluid was made by mixing together mercuric chloride 0.5 g, sodium chloride 6.5 g, hydrochloric acid 1.25 ml, absolute alcohol 500 ml and water to make 1000 ml. The eggs were treated in this fluid for 20 min followed by 35 min exposure to sterile 70% ethanol and then finally washed in sterile water for at least 5 min. In this way egg-surface could be sterilized without any ill effect on the eggs. A sterility test was always run by inoculating sterilized whole eggs as such in the nutrient medium and incubating at 37°C for 24 h after which an absence of contamination indicated the successful surface sterilization. Another batch of similarly treated eggs was macerated in sterile water and the suspension was transferred aseptically into the culture medium. After 24 h of incubation at 37°C, the culture medium was full of growing symbiotes of similar type as that found in the egg-smear (Fig. 1). The cultivated symbio-

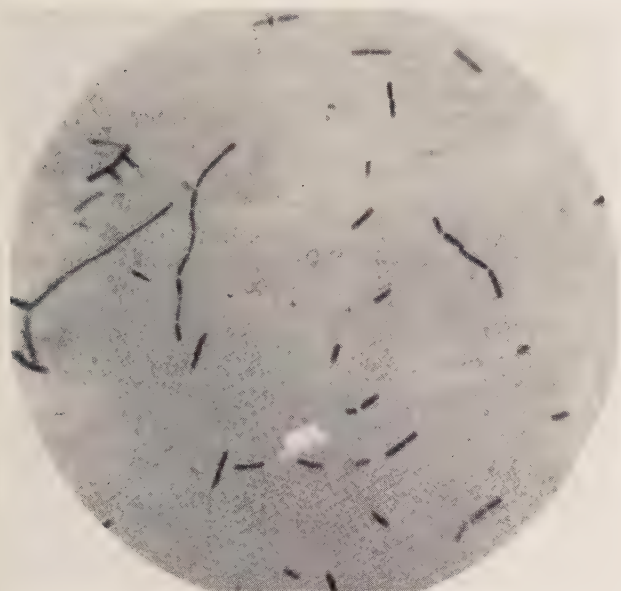


Fig. 2.—Photomicrograph of cultivated symbiotes of *O. surinamensis*.

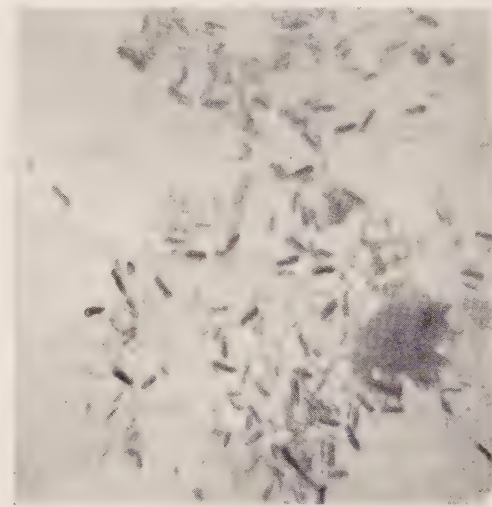


Fig. 1.—Photomicrograph of egg smear of *O. surinamensis* showing bacterium like symbiotes.

aspects of the symbiosis and concluded that the symbiotes may be supplying some of the accessory growth factors.

Some attempts are reported to have been made to cultivate the symbiotes outside the body of the insect in artificial media, but they were never successful³. We have now been able to cultivate successfully the bacterium-like symbiotes of *Oryzaephilus surinamensis* on lactosenutrient broth consisting of the following ingredients:

Beef extract	3.0 g
Peptone	5.0 g
Lactose	5.0 g
Distilled water	1000.0 ml
pH adjusted to 7-7.2	

To this medium was added the inoculum containing symbiotes obtained from eggs. The eggs were externally sterilized after the manner described by BEGG and SANG⁴.

tes are 2-4 $\mu \times$ 2-3 μ in size and gram negative (Fig. 2). After 30 h of incubation, they start forming long chains.

We thank Prof. M. L. BHATIA for suggestions and giving research facilities in his Department. Thanks are due to Dr. B. D. SANWAL, Department of Botany, for his very valuable help in the bacteriological methodology and also to Mr. E. A. DENIELS for taking the microphotographs presented here.

N. C. PANT*, J. K. NAYAR, and Miss P. GUPTA

Department of Zoology, University of Delhi (India), February 25, 1957.

Zusammenfassung

Bakterienähnliche Symbionten von Eiern der *O. surinamensis* sind auf künstlichem Laktose-Nährboden mit Erfolg gezüchtet worden. Die Zuchtformen sehen gleich aus wie die Symbionten der Eier.

¹ U. PIERANTONI, R. C. Accad. Lincei 9, 451 (1929); Atti Accad. Sci. Fis. Mat. Napoli 18, 1 (1930).
² A. KOCH, Z. Morph. Ökol. Tiere 23, 389 (1931); 32, 137 (1936).
³ N. C. PANT and G. FRAENKEL, J. zool. Soc. India 6, 173 (1954).
⁴ M. BEGG and J. H. SANG, Science 112, 11 (1950).

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The Semi-quantitative Determination of Cholesterol and Cholesteryl Esters by Paper Chromatography

In previous papers we described the one-dimensional separation of cholesterol and its esters¹. We mentioned the possibility of the semi-quantitative determination. Further details of this procedure are given here:

To 1 ml of blood serum 9 ml of ethanol-ether mixture 3:1 (v/v) were added. After 10 min standing, the mixture was centrifuged and 5 ml of clear supernatant were evaporated to dryness in a test tube. The residue having been extracted with 0.1 ml of chloroform, 0.04 ml of this solution were spotted on Whatman No. 3 paper impregnated with paraffin oil². The mixture of acetic acid-chloroform-paraffin oil 65:25:10 (v/v/v) was used as a mobile phase. After 12–15 h of ascending run, the chromatogram was dried and cut into strips 1 cm wide and these eluted with 3 ml of chloroform for 5–6 h at room temperature, in test tubes with occasional stirring. Then 1 ml of acetic anhydride and 0.1 ml of concentrated sulphuric acid were added and the density of the coloured solution was measured at 610 m μ after 10 min.

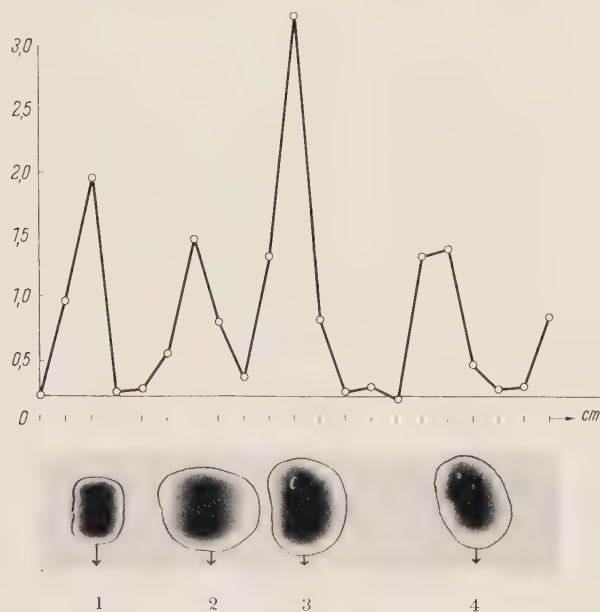


Fig. 1.—Mixture of synthetic cholesteryl esters. 1 Cholesteryl oleate; 2 Cholesteryl caproate; 3 Cholesteryl formate and acetate; 4 Cholesterol.

The calibration curve was prepared from the standard solution of cholesterol. The calculation was carried out by addition of all extinction values of every individual spot and subtraction of the blanks and then multiplication with the factor calculated from the calibration curve.

For the calculation of only relative percentage, it is possible to weigh the individual pieces of paper obtained by cutting out the individual peaks of the graph representing relation between the extinction values and the distances in cm.

The results are in good agreement with those obtained by other methods.

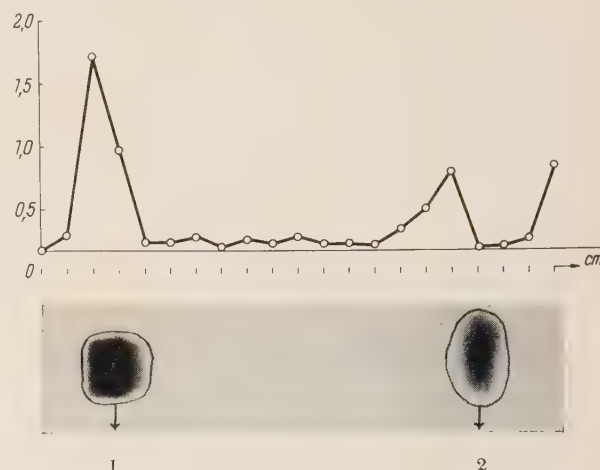


Fig. 2.—Human blood serum. 1 Cholesteryl esters with higher fatty acids (oleate, palmitate, linoleate); 2 Free cholesterol.

The present method is simple and permits a comparatively rapid determination of free and esterified cholesterol in blood serum and other biological fluids and organs.

Č. MICHALEC, V. JIRGL, and
J. PODZIMEK

Central Biochemical Laboratories of the University
Hospital, Prague (Czechoslovakia), February 8, 1957.

Zusammenfassung

Eine papierchromatographische Methode zur semi-quantitativen Bestimmung von Cholesterin und Cholesterinester in biologischem Material wird beschrieben.

Application vaginale de cystamine et Radio-Protection locale

I. *Introduction.* Dans un travail antérieur publié dans cette même revue, l'un de nous¹ a pu montrer que l'administration de Becaptan par voie générale protège la muqueuse vaginale de la rate contre les effets d'une irradiation locale.

Les travaux expérimentaux consacrés aux radio-protecteurs en général et au Becaptan en particulier ont été le plus souvent réalisés en administrant la substance protectrice par voie générale; l'effet est alors étudié soit sur l'organisme *in toto* (courbes de mortalité, chute de poids) soit au niveau d'un tissu déterminé (ovaire, intestin grêle, foie, rate, thymus, lignée rouge, leucocytes, muqueuse vaginale etc.).

Peu de recherches ont été consacrées à l'action protectrice de l'application locale d'une substance déterminée au niveau de l'organe à protéger. Nous pouvons cependant citer:

¹ Č. MICHALEC, *Naturwissenschaften* 42, 509 (1955).

² Č. MICHALEC, *Biochim. biophys. Acta* 19, 187 (1956).

¹ L. DARCIS, P. HOTTERBEECH et C. ONKELINX, *Exper.* 12, 286 (1956).

Pourcentages des lésions cellulaires observées dans les jours qui suivent l'irradiation pendant 4 heures de rates protégées localement par la cystamine

Jours	Rates									
	1	2	3	4	5	6	7	8	9	10
4	17	18	14	—	20	—	19	—	25	—
5	16	20	17	15	27	24	26	17	21	18
6	19	25	23	19	29	17	22	27	16	21
7	20	15	20	22	18	15	14	12	13	—
8	—	16	18	16	15	10	16	9	—	15
9	11	10	19	—	12	8	11	8	10	—
10	8	—	10	11	—	7	8	5	9	8

1° FORSSBERG² qui a pu démontrer l'action protectrice locale de la cystéine, en prenant comme test l'épilation de la peau du cobaye sous l'effet des rayons X; la cystéine était administrée par la voie intra-dermique.

2° BACQ et HERVÉ³ qui, dans des travaux encore inédits, ont mis en évidence que la régénération des poils est favorisée par l'application d'une pommade à base de cystamine (2% de cystamine dans du Carbowax) préalablement à l'irradiation de la peau rasée, chez le rat.

Le présent travail étudie l'action protectrice, au niveau du vagin de la rate de la cystamine administrée *in loco*.

II. *Techniques et méthodes.* Nous ne ferons que les résumer.

1° L'irradiation locale est réalisée au moyen d'un tube de radium que l'on introduit dans la cavité vaginale, sous anesthésie générale, et dont les caractères physiques sont: longueur totale: 16 mm; longueur active: 12 mm; diamètre extérieur: 4 mm; diamètre intérieur: 2 mm; filtration: 1 mm Pt., charge: 50 mg radium.

De cette façon, la dose reçue par la muqueuse vaginale est d'environ 2275 r · γ/h. L'anesthésie est prolongée pendant toute la durée de l'irradiation pour éviter l'expulsion du tube; au cours de cette série d'expériences, chaque irradiation a duré 4 h.

2° L'étude des frottis vaginaux (colorés par la méthode de Papanicolaou) prélevés quotidiennement pendant les 15 jours qui suivent l'irradiation met en évidence l'existence de cellules radio-lésées. Nous considérons comme cellule radio-lésée toute cellule nucléée dont le plus grand diamètre dépasse 45 μ ou qui renferme plusieurs noyaux.

3° Pour une même durée d'irradiation, le pourcentage de cellules radiolésées varie en fonction du temps qui sépare l'irradiation du prélèvement du frottis.

a) Il existe constamment une période de latence s'étendant habituellement sur 3 à 5 jours, au cours desquels le pourcentage d'altérations cellulaires reste dans la limite de la normale (un frottis vaginal normal ne renferme pas plus de 10% de cellules présentant les caractères définis plus haut).

b) Les cellules radio-lésées apparaissent dans les frottis du 4^e au 6^e jour; leur pourcentage passe par un maximum entre le 6^e et le 10^e jour puis décroît pour reprendre, entre le 8^e et le 15^e jour, voire au delà, des valeurs normales.

4° L'application locale de cystamine a été réalisée par une irrigation continue au moyen d'une solution aqueuse renfermant 10% de cystamine. Cette irrigation fut faite au moyen d'un très fin tube de polythène introduit dans

le vagin de la rate et raccordé à un petit «Baxter» de fortune (flacon à pénicilline modifié pour la circonstance) rempli de la solution. Cette irrigation est mise en train ½ h avant le début de l'irradiation et se prolonge pendant toute la durée de celle-ci (durée totale d'application: 4 ½ h), ce qui entraîne l'écoulement d'environ 5 cm³ de la solution dans le vagin. Pour chaque rate, la solution a été préparée extemporanément.

5° Enfin, les animaux utilisés sont des rates vierges provenant de l'élevage sélectionné du Centre Anticancéreux près l'Université de Liège. Chacune pesait au départ, environ 150 g.

III. *Résultats.* Trois groupes d'animaux ont été étudiés:

1° 18 animaux ont été irradiés pendant 4 h, sans administration de protecteur: les pourcentages maxima de cellules radio-lésées oscillent entre 45 et 70%, soit en moyenne 60 ± 2%.

2° Rappelons les résultats obtenus chez 13 rats ayant reçu 15 mg de Becaptan par la voie intra-péritonéale immédiatement avant l'irradiation; dans ce groupe d'animaux, les pourcentages maxima de cellules radio-lésées oscillent entre 20% et 34%, en moyenne 27 ± 3%.

3° Enfin, le Tableau rassemble les résultats obtenus chez 10 rates auxquelles nous avons administrés localement de la cystamine suivant la technique décrite plus haut.

Nous voyons que les pourcentages maxima oscillent entre 20% et 29%, en moyenne 24 ± 1%. Il ressort de cette expérience que la protection vaginale obtenue par l'administration locale de cystamine est équivalente à celle obtenue par l'administration intra-péritonéale de cystéamine.

Il reste à déterminer si cette protection est strictement locale ou si, résorbé en quantité suffisante, le protecteur a été lancé dans la circulation générale comme au cours d'une administration intra-péritonéale. Nous pensons pouvoir donner prochainement une solution à ce problème.

L. DARCIS et G. GILSON

Centre Anticancéreux de l'Université de Liège (Belgique),
le 27 décembre 1956.

Summary

In a previous paper, the authors showed that intraperitoneally administered cystamine protects the vaginal mucosa of rats against local irradiation.

Using the same technique (study of radiation-lesions in vaginal smears), they demonstrate that locally administered cystamine is also effective.

2 A. FORSSBERG, Acta radiologica 33, 296 (1950).
3 Z. BACQ et A. HERVÉ, Communication personnelle.

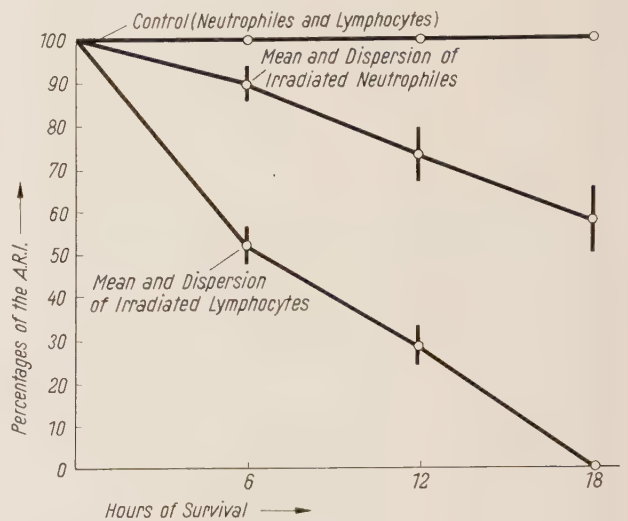
Experimental Investigations on the Influence of X-Rays on Glycogen Content in Surviving Leucocytes*

With regard to the influence that antimitotic agents may have upon normal tissues and cells during anti-neoplastic therapy, we have studied the influence of X-rays on the glycogen content of leucocytes.

The radiation was carried out directly on the cells surviving *in vitro*. In fact, during survival leucocytes keep their biological activities for a time, including the metabolic ones. In particular, with reference to the glycogen metabolism, glycogenolysis occurs in leucocytes during survival and they utilize their glycogen content (ASTALDI, BERNARDELLI, RONDANELLI¹). Moreover, when leucocytes survive in a medium enriched in glucose, glycogenesis occurs and, as a consequence, leucocytes increase their glycogen content (ASTALDI, BERNARDELLI, RONDANELLI, and GORINI²). On the other hand, X-rays can diminish the A.P.S. positive structures of the connective tissue when patients are irradiated (PISANI and ROMANINI³), and also some cytotoxic substances can interfere with the glycogen content of the liver cells and of leucocytes, too (CARDINALI, CAPRINO and PISCITELLI⁴). Finally, regarding the influence of X-rays on the biological properties of surviving leucocytes, ASTALDI and MASSA⁵ have shown an increase in their osmotic fragility.

* These researches have been performed with the help of the «Lega italiana per la lotta contro i Tumori».
¹ G. ASTALDI, E. BERNARDELLI, and E. G. RONDANELLI, Boll. Soc. Ital. Biol. sper. 28, 286 (1952).
² G. ASTALDI, E. BERNARDELLI, E. G. RONDANELLI, and P. GORINI, Boll. Soc. Ital. Ematol. 3, 87 (1955).
³ G. PISANI and A. ROMANINI, Tumori 40, 410 (1954).
⁴ G. CARDINALI, G. CAPRINO, and M. PISCITELLI, Gazz. int. Med. Chir. 59, 1331 (1954); Tumori 40, 176 (1954).
⁵ G. ASTALDI, 6th International Congress of Haematology (Boston 1956). – G. ASTALDI and B. MASSA, to be printed.

The researches were carried out on leucocytes from normal human beings (10 subjects). Leucocytes were kept in homologous serum at 37°C, and a part was kept as control, another was irradiated with 1000 r. A big dose like this was given to increase the probability of seeing the effect, if any.



Glycogen content of irradiated leucocytes in relation to the control (control = 100).

The irradiation was made according to the following conditions: unic dose, kV 140, mA 10, filter 3 Al, distance cm 14, r/m' 600. At this point, special thanks are due to Dr. B. UGGERI and Dr. A. GIORDANO from the Hospital of Tortona, for their radiological assistance.
At the beginning of the experiment, and periodically to the 6th, 12th and 18th h of survival, samples of leucocytes were taken and smeared. To demonstrate the

Table I.—Neutrophils

Hours after irradiation	Cases										Mean and Dispersion	't' Student			
												Calculated	Snedecor's		
	1	2	3	4	5	6	7	8	9	10	$m \pm \sigma_m$				
0	3.9	3.8	4.0	4.0	3.8	3.9	3.7	3.9	5.0	4.0	3.9	3.3 ± 0.20 2.95 ± 0.017	6.92	P=5%	P=1%
6	3.5	3.6	3.4	3.3	3.1	3.5	3.7	3.5	3.0	3.3					
	3.1	3.2	3.0	3.2	2.8	2.8	2.9	3.2	2.5	2.8					
12	2.8	3.2	2.9	2.7	2.5	2.9	2.7	2.8	3.0	2.6	2.75 ± 0.2	1.95 ± 0.2	9.74	2.26	3.25
	2.2	2.0	1.9	2.1	2.1	1.8	2.1	2.0	2.2	1.9					
18	1.5	1.0	1.4	1.4	0.9	1.5	1.3	1.2	1.4	1.6	1.32 ± 0.07	0.79 ± 0.07	9.64	2.26	3.25
	1.2	0.4	0.8	1.1	0.5	0.7	0.9	0.7	0.8	0.9					

Table II.—Lymphocytes

Hours after irradiation	Cases										Mean and Dispersion	't' Student			
												Calculated	Snedecor's		
	1	2	3	4	5	6	7	8	9	10	$m \pm \sigma_m$				
0	0.8	0.7	0.5	0.8	0.8	0.6	0.7	0.9	0.8	0.6	0.72	6.63	2.26	3.25	
6	contr. . . .	0.5	0.4	0.3	0.6	0.6	0.4	0.5	0.6	0.4	0.3				0.46 ± 0.04
	irrad. . . .	0.3	0.15	0.15	0.3	0.3	0.2	0.15	0.3	0.25	0.2	0.23 ± 0.003			
12	contr. . . .	0.1	0.15	0.10	0.40	0.20	0.20	0.15	0.10	0.20	0.20	0.18 ± 0.008	3.60	2.26	3.25
	irrad. . . .	0.0	0.05	0.03	0.20	0.10	0.0	0.05	0.0	0.10	0.0	0.053 ± 0.004			
18	contr. . . .	0.0	0.10	0.0	0.20	0.05	0.0	0.05	0.0	0.05	0.05	0.05 ± 0.004	2.44	2.26	3.25
	irrad. . . .	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0			

glycogen in cells, the smears were submitted to the P.A.S. reaction, according to HOTCHKISS. To valuate the average glycogen content per cell, the average reaction index (A.R.I.) according to ASTALDI *et al.*⁶ was determined separately for neutrophils and lymphocytes.

The results obtained from neutrophils are given in Table I, those regarding lymphocytes in Table II. The Figure expresses graphically the quantitative behaviour of the glycogen content of the irradiated leucocytes in relation to the control taken as 100.

The differences between the control and the irradiated leucocytes were submitted to statistical analysis, using Student-Fisher *t* test. The differences resulted in a significant degree, applying the *t* value to the differences in pairs.

From the results taken as a whole, we may conclude that X-rays carried out directly on surviving leucocytes cause an evident increase of glycogenolysis in these cells. Such a phenomenon occurs to a still more marked degree in lymphocytes than in neutrophils.

G. ASTALDI and L. VERGA

Department of International Medicine, University of Pavia, and The Blood Research Foundation Center, Hospital of Tortona (Italy), December 27, 1956.

Riassunto

Sono stati irradiati direttamente *in vitro* leucociti sopravvivenuti ed è stato studiato il loro contenuto in glicogeno durante la sopravvivenza.

I risultati dimostrano un aumento significativo della glicogenolisi nei leucociti irradiati rispetto ai controlli. Riguardo alla perdita del loro contenuto in glicogeno, i linfociti risultano più sensibili dei neutrofili alla irradiazione Roentgen.

⁶ G. ASTALDI, E. BERNARDELLI, and E. G. RONDANELLI, Haematology 36, 749 (1952).

The Influence of Lowering the Body Temperature on Postural Orientation of the Organism

It is well known that the postural orientation of the organism as a whole and of separate parts of the body is realized by the righting reflexes of Magnus, the centres of which are localized in the reticular formation of the brain-stem at the level of the nucleus ruber. Stereotaxic lesions are a common cause of elimination of these reflexes. This causes irreversible damage, which usually effects simultaneously the centres of several reflexes. It is possible to obtain a successive, and at the same time a relatively quick, functional elimination of these reflex centres, which is reversible, and which was obtained in our experiments by a lowering of the body temperature.

A considerable drop of temperature of the environment was used for lowering the animal body temperature (30 rabbits). The loss of a prompt and exact body righting response was denoted as the stage of a decreased response. As can be seen from the Table, lowering of the body temperature first leads to the extinction of Magnus' body righting reflexes acting on body, while the labyrinthine righting reflexes are the last to be lost. The recovery of reflexes takes place in the reverse order, i.e. first to appear, are the labyrinthine reflexes. The order

The order in which the postural reflexes are eliminated after lowering the body temperature

Rectal temperature °C	Percentage of animals, in which a postural reflex response was seen					
	I		II		III	
	a	b	a	b	a	b
25–24	30					
23–22	70	20	30	10	10	
21–20	20	80	60	40	30	20
19–18		100	10	90	40	50
17–16				100	20	80
15–14						100

I Body righting reflexes acting on body (Magnus)
II Neck righting reflexes
III Labyrinth righting reflexes
a Decreased reflex response, b Extinction of reflexes.

in which the elimination of reflexes progresses is quite constant and also stresses the importance of the labyrinths for spatial analysis, which makes possible the general postural orientation of the organism.

D. SVORAD

Institute of Physiology, Czechoslovak Academy of Sciences, Prague, December 20, 1956.

Zusammenfassung

Durch starke Unterkühlung des Warmblüterorganismus kann man die Stellreflexe sukzessiv und reversibel ausschalten. Zuerst erlöschen die Magnusschen Körper-Stellreflexe und zuletzt die labyrinthären Stellreflexe.

Versuche zur biologischen Bestimmung der Sedimentationskonstanten kleiner neurotroper Viren in der präparativen Ultrazentrifuge «ZF 3»

BEYERLE, MOHRING und BÜCHER¹ berichteten 1954 über eine neue, elektromagnetisch angetriebene Präparierzentrifuge (Typenbezeichnung «ZF 3»), deren besondere Eigenart darin besteht, dass das gesamte Einsatzvolumen in einer einzigen, radial zur Drehachse gerichteten Zelle zusammengefasst ist. Ein Gerät dieser Bauart wurde in unserem Laboratorium erstmalig für virologische Studien eingesetzt und hat sich seit längerer Zeit beim präparativen Arbeiten mit kleinen neurotrophen Viren bewährt. Die dabei gesammelten Erfahrungen veranlassen uns zu prüfen, ob die Zentrifuge auch zur Bestimmung von Sedimentationskonstanten auf biologischem Wege, das heisst aus der Aktivitätsabnahme im Überstand der Zelle, herangezogen werden kann. Als Modell für unsere Untersuchungen wählten wir das Encephalomyocarditis-(EMC-)Virus der Maus, dessen Sedimentationskonstante von WEIL *et al.*² mittels

¹ K. BEYERLE, D. MOHRING und Th. BÜCHER, Chem. Ing. Technik 26, 94 (1954).
² M. L. WEIL, J. WARREN, S. S. BREESE, S. B. RUSS und H. JEFFRIES, J. Bacteriol. 63, 99 (1952).

der Lichtabsorptionsmethode zu 148 bis 159 S bestimmt wurde.

Material und Methoden. Aufbau und Arbeitsweise der Zentrifuge wurden bereits ausführlich von BEYERLE *et al.*¹ beschrieben. Zum besseren Verständnis des folgenden sei lediglich auf die Radialzelle (Abb. 1) noch einmal kurz eingegangen. Sie besteht aus einem mechanisch und chemisch besonders widerstandsfähigen Stahl, verfügt über ein Fassungsvermögen von etwa 6 cm³ und gestattet bei annähernd doppelter Werkstoffsicherheit Drehzahlen bis zu 84 000 U./min (= 500 000 g am Zellenboden). In den zylindrischen Präparatraum können verschiedene, aus Plexiglas angefertigte Sedimentfallen eingesetzt werden, die zum Erfassen des Sediments sowie als Schutz gegen Strömungen und Diffusion beim Abbremsen der Zentrifuge dienen. Besonders bewährt hat sich ein auch für die folgenden Versuche benutzter Fallentyp, bei dem der Fallinhalt (0,04 cm³) durch einen Düsentrichter von dem übrigen Zellvolumen abgetrennt ist.

Als Untersuchungsmaterial verwendeten wir 1%ige Homogenate infektiöser Mäusegehirne in m/15 Phosphatpuffer pH 7,4. Größere Zelltrümmern wurden durch 20 min langes Zentrifugieren bei 15 000 U./min abgeschleudert. Die Zentrifugationen in der ZF 3 erfolgten zu gestaffelten Versuchszeiten bei 56 000 U./min (± 200). Die Anlaufzeiten bis zum Erreichen der vollen Drehzahl betrugen etwa 85, die Bremszeiten zwischen 200 und 250 s. Durch Kühlung der Läuferkammer mit zirkulierender Kältesole (-20°C) und ständiges Spülen mit Wasserstoffgas (10 Torr) konnte die Temperatur der entsprechend vorgekühlten Zelle von Beginn bis Ende eines Laufes auf 6°C ($\pm 0,2$) begrenzt werden.

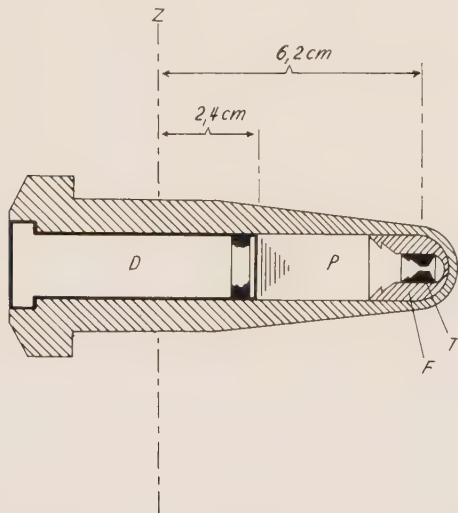


Abb. 1. Radialzelle der Präparierzentrifuge ZF 3. Z = Drehachse, P = Präparatraum, D = vakuumdichter Verschlusskörper, F = Sedimentfalle mit Düsentrichter (T).

Nach der Zentrifugation wurde jeweils der gesamte Überstand oberhalb des Düsentrichters abpipettiert, durchmischt und auf seine Infektiosität (LD_{50} nach REED und MUENCH³) hin geprüft. Die Titrationen erfolgten durch Verimpfen von 10fachen Verdünnungsreihen auf etwa 14 g schwere weiße Mäuse. Pro Verdünnungsstufe wurden jeweils 6 Tiere subkutan mit je 0,2 ml infiziert. Die Titerdifferenzen zwischen dem Ausgangsmaterial und den Überständen liefern einen Massstab für die während der Zentrifugation stattgefundenen

Konzentrationsabnahmen, aus denen die Sedimentationskonstante des Virus errechnet werden soll.

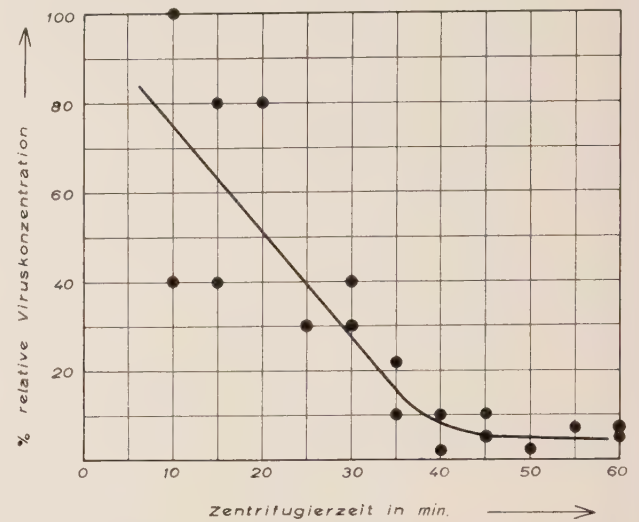


Abb. 2. Aktivitätsabnahme im Zellenüberstand (56 000 U./min, 6°C).

Ergebnisse. In Abbildung 2 haben wir die relativen Viruskonzentrationen der Überstände (bezogen auf das Ausgangsmaterial = 100%) gegen die Versuchszeiten aufgetragen. Die aus den einzelnen Punkten konstruierte Kurve veranschaulicht den Sedimentationsvorgang. Im einzelnen zeigt sie, dass bis zu einer Zentrifugierdauer von etwa 40 min eine im Durchschnitt gesehen kontinuierliche Konzentrationsabnahme stattfindet. Im weiteren Verlauf flacht die Kurve ziemlich plötzlich ab und geht in einen fast parallel zur Abszisse auslaufenden Abschnitt über. Es verbleiben also im Überstand gewisse Restaktivitäten, die zwischen 1–10% der Ausgangsinfektiosität betragen. In einem ergänzenden Versuch wurde der nach 60 min langer Zentrifugierdauer immer noch infektiöse Überstand ein zweites Mal über 40 min zentrifugiert. Hierbei zeigte sich erneut eine Konzentrationsabnahme von mehr als 90%. Die Restaktivitäten werden demnach nicht durch kleinere Einheiten des infektiösen Prinzips hervorgerufen, sondern müssen auf methodisch bedingte Faktoren zurückgeführt werden.

Für das Berechnen der Sedimentationskonstante haben wir die von SVEDBERG und PEDERSEN⁴ für sektorförmige Separationszellen abgeleitete Gleichung auf die zylindrische Gestalt unserer Zelle umgeformt. Die Gleichung lautet dann

$$S = - \frac{1}{\omega^2 \cdot t} \cdot \ln \left[\frac{x_0}{x_i} + \frac{C_t \cdot (x_i - x_0)}{C_0 \cdot x_i} \right]$$

(ω = Winkelgeschwindigkeit, t = Zentrifugierdauer, x_0 = Abstand zwischen Drehzentrum und Meniskus, x_i = Abstand zwischen Drehzentrum und Zellenboden, wobei wir als Zellenboden die Mitte des Düsentrichters gewählt haben, C_0 = Konzentration (Aktivität) im Ausgangsmaterial, C_t = Konzentration im Überstand nach der Zentrifugation.)

Die Formel berücksichtigt die Inhomogenität des Schwerfeldes, die in der ZF 3 besonders gross ist, da hier die effektive Zelllänge ungewöhnlich lang, die Abstände des Meniskus und Zellenbodens vom Drehzen-

³ L. J. REED und H. MUENCH, Amer. J. Hyg. 27, 493 (1938).

⁴ T. SVEDBERG und K. O. PEDERSEN, Die Ultrazentrifuge (Verlag Steinkopf, Leipzig und Dresden 1940).

trum dagegen nur kurz sind. Anlauf- und Bremszeiten wurden wegen ihrer Kürze im Verhältnis zur Zentrifugierdauer bei voller Drehzahl nicht berücksichtigt. Die möglichen Fehlerquellen, die dadurch zu erwarten sind, dass die Zellform nicht der radialen Wanderungsrichtung der sedimentierenden Teilchen angepasst ist, seien zunächst einmal vernachlässigt.

Aktivitätsabnahmen und daraus errechnete Sedimentationskonstanten des EMC-Virus bei Zentrifugerversuchen in der ZF3 (56000 U./min, 6°C)

Zentrifugierzeit in s	Versuch Nr.	-log LD ₅₀		log Titerdifferenz	C _t /C ₀	S ₂₀
		V	N			
600	1	7,3	7,5	—	—	—
600	2	7,7	7,3	0,4	0,4	349
900	3	7,3	7,2	0,1	0,8	71
900	4	7,7	7,3	0,4	0,4	232
1200	5	7,3	7,2	0,1	0,8	53
1500	6	7,5	7,0	0,5	0,3	169
1800	7	7,7	7,3	0,4	0,4	118
1800	8	7,5	7,0	0,5	0,3	141
2100	9	7,7	7,0	0,7	0,2	137
2100	10	7,3	6,3	1,0	0,1	175
2400	11	7,5	6,5	1,0	0,1	153
2400	12	7,7	6,0	1,7	0,02	175
2700	13	7,5	6,5	1,0	0,1	137
2700	14	7,3	6,0	1,3	0,08	145
3000	15	7,7	6,2	1,5	0,03	137
3300	16	7,3	6,2	1,1	0,08	116
3600	17	7,3	6,0	1,3	0,05	108
3600	18	7,3	6,2	1,1	0,08	106
Mittelwert aus den Versuchen 6 bis 14						150

V = im Ausgangsmaterial vor der Zentrifugation
N = im Überstand nach der Zentrifugation

In der Tabelle sind die Daten aller Einzelversuche und die daraus errechneten Sedimentationskonstanten (in Svedbergeinheiten, bezogen auf Wasser als Lösungsmittel bei 20°C) zusammengestellt. Legt man den von WEIL *et al.*² auf optischem Wege gefundenen Mittelwert von 154 S als Maßstab für die Genauigkeit unserer Ergebnisse zugrunde, so zeigt sich, dass die Versuche mit einer Zentrifugierdauer von 25 bis 50 min, das heisst Versuche im Bereich einer durchschnittlichen Aktivitätsabnahme von etwa 70 bis gerade 90%, die besten Resultate liefern. Die Einzelwerte stimmen befriedigend, der Mittelwert sogar überraschend gut mit der optisch bestimmten Sedimentationskonstante überein. Beim Zentrifugieren über jenen Zeitpunkt hinaus, zu dem eine durchschnittliche Konzentrationsverminderung um 90% erreicht ist, werden die errechneten S-Werte zunehmend kleiner. Versuchszeiten im Bereich einer durchschnittlichen Aktivitätsabnahme von weniger als 70% ergeben übermässig streuende Sedimentationskonstanten.

Diskussion. 1. Die Zentrifuge ZF 3 kann, obgleich die Seitenwände der Zelle und die Aussenteile der Sedimentfalle nicht der radialen Bewegungsrichtung der Partikel angepasst sind und das herrschende Schwerfeld recht inhomogen ist, zur biologischen Bestimmung der Sedimentationskonstanten tierpathogener Viren herangezogen werden. Voraussetzung ist, dass der Sedimentationsvorgang analysiert wird und nur solche Einzelversuche ausgewertet werden, die hinsichtlich der

Zentrifugierdauer im Bereich einer durchschnittlichen Aktivitätsabnahme von etwa 70 bis gerade 90% liegen.

2. Versuche mit kürzeren Laufzeiten führen zu einer selbst für biologische Methoden kaum mehr vertretbaren Streuung der errechneten Sedimentationskonstante. Die Ursache hierfür ist vornehmlich darin zu suchen, dass sich die begrenzte Güte des biologischen Tests bei geringen Aktivitätsabnahmen, das heisst bei kleinen C_t/C₀-Werten, zunehmend nachteilig auswirkt. Aus dem gleichen Grunde streuen auch die nach kurzen Laufzeiten erhaltenen Punkte in Abbildung 2 besonders stark.

3. Zentrifugiert man andererseits über den Zeitpunkt der erreichten 90prozentigen Aktivitätsabnahme hinaus, so errechnen sich zu kleine S-Werte. Es ist dies darauf zurückzuführen, dass der Sedimentationsvorgang bei einer Konzentrationsverminderung um etwa 90% bis auf das Verbleiben gewisser Restaktivitäten nahezu beendet ist und ein überschüssiges Zentrifugieren somit nicht mehr dem theoretischen Anwendungsbereich der oben genannten Gleichung entspricht.

4. Konvektionsströmungen, die infolge der zylindrischen Zellform und der Sedimentfalle zu erwarten sind, können nur so geringfügig sein, dass hierdurch die ohnehin begrenzte Exaktheit biologischer S₂₀-Bestimmungen nicht beeinträchtigt wird. Die Restaktivitäten sind nicht oder zumindest nicht ausschliesslich auf die Gestalt der Zelle zurückzuführen. Das gleiche Phänomen findet sich auch beim Arbeiten mit sektorförmigen Zellen (EPSTEIN und LAUFFER⁵, DREES⁶).

5. Es muss dahingestellt bleiben, wie sich die Viruspartikel, die sich ausserhalb des der radialen Wanderungsrichtung entsprechenden Zellvolumens befinden, während der Sedimentation verhalten. Da die Menge etwa 1/4 des gesamten Einsatzvolumens ausmacht, könnte durch sie das Ergebnis nur dann merklich beeinträchtigt werden, wenn dieses Virus nach Erreichen der Zellwand dort haften bleibt und beim Abpipettieren annähernd quantitativ wieder in den Überstand eingeht. Das aber ist, gemessen an unseren Befunden, offensichtlich nicht der Fall. Ein beschleunigtes Absinken der an den Zellwänden angelangten Viruspartikel ist für die Auswertung bei 70- bis 90prozentiger Aktivitätsabnahme belanglos, da die Menge des hierfür in Betracht kommenden Virusanteils zu klein ist, um das Ergebnis überhaupt beeinflussen zu können.

O. DREES und P. BOESCHE

Laboratorium der Stiftung zur Erforschung der spinalen Kinderlähmung und der Multiplen Sklerose, Hamburg-Eppendorf, den 2. März 1957.

Summary

An attempt is made to determine the sedimentation constant by means of biological assay in animal viruses. A new type of electromagnetic high speed centrifuge (Type ZF 3) was used for this purpose. An essential feature of this model is that the entire volume for assay is included in one single cell positioned at an angle of 90° to the axis of rotation. The murine EMC virus was used to show that the centrifuge is suitable for the biological determination of S-values, in spite of the fact that the gravitational field in the cell is inhomogeneous and that the cell walls are not adapted to the radial migrational direction of the centrifuged particles.

⁵ H. T. EPSTEIN und M. A. LAUFFER, Arch. Biochem. Biophys. 36, 371 (1952).

⁶ O. DREES, erscheint demnächst in Kolloid-Z.

Amino Acid Studies in Relation to Yolk Utilization in the Chick Embryo

EMANUELSSON¹ has demonstrated three separate proteolytic enzymes in the yolk of the chick egg by incubation procedures *in vitro* and WILLIAMS *et al.*², using paper chromatography, have reported an increase of free amino acids of whole yolk from the fourth to the 10th day of incubation. It seemed important to supplement the existing knowledge concerning the amino acid pools of the hen's egg by assaying the level of free amino acids in the solid yolk, fluid yolk and blood between 3 and 12 days of incubation.

Materials and Methods. Samples of blood were collected at 7, 9, and 12 days by isolating a section of a vitelline artery over a small plastic 'boat', and after carefully cleaning the vessel of investing tissue, the artery could be cut and the 'clean' blood collected from the plastic boat. From 3 until 10 days of incubation in the chicken egg there is a volume of liquid (sub-blastodermic fluid or fluid yolk), containing soluble protein, which lies beneath the embryo and above the solid yolk (ROMANOFF³). This fluid was collected by means of small bore mouth pipets and it was then centrifuged at 18,000 g for 10 min to free it of yolk granules. Trichloroacetic acid was added to the solid yolk, liquid yolk and blood to give a final concentration of 10%.

Since the object of this investigation was to determine total free amino acid content in these various fractions the use of Folin's amino acid reagent for the colorimetric

estimation of amino nitrogen (KOCH and HANKE⁴) seemed quite adequate. Total nitrogen determinations were made utilizing the nesslerization procedure described in UMBREIT *et al.*⁵.

Experimental Results. To give an idea of the rate of growth, embryo dry weights were obtained and these are given in the Table; embryos were pooled in obtaining the dry weights of the early stages. Also in the Table the total nitrogen values of TCA precipitates from 5 cm³ samples of fluid yolk during this same period are indicated. At 3 days of incubation approximately 5 cm³ of liquid yolk could be obtained from the yolk sac, at 4 days there was about 10 cm³ and approximately 15 cm³ at 6 and 8 days; the volume decreases after this time.

In the series of analyses for amino nitrogen in fluid yolk samples the data show an amino acid increase during development (Table), and it is probable that this increase results from proteolysis of yolk protein, although these amino acids could have another source (albumin, embryo or its membranes). Based on equal sample volumes, the free amino acids maintained a constant concentration in the blood and the solid yolk during the period from 3 to 12 days of incubation (Table).

Acknowledgment. This work was aided by a grant from the Committee on Growth Acting for the American Cancer Society and a University of California Cancer grant.

R. A. FLICKINGER

Department of Zoology, University of California, Los Angeles, October 29, 1956.

¹ H. EMANUELSSON, *Acta physiol. Scand.* 34, 124 (1955).
² M. A. WILLIAMS, W. A. DA COSTA, L. H. NEWMAN, and L. H. MARSHALL, *Nature* 173, 490 (1954).
³ A. ROMANOFF, *The avian egg*. (John Wiley and Sons, Inc., 1949).

⁴ F. C. KOCH and E. HANKE, *Practical methods in biochemistry* (The Williams and Wilkins Co., 1948).
⁵ W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric techniques and related methods for the study of tissue metabolism* (Burgess Publishing Company, 1948).

Days of development	Embryo dry weight mg	Fluid yolk acid insoluble nitrogen in 5 cm ³ samples μg	Fluid yolk amino nitrogen in 5 cm ³ samples μg	Whole blood amino nitrogen in 1 cm ³ samples μg	Solid yolk amino nitrogen in 5 cm ³ samples μg
3		2,700 2,800 2,706	1,025 1,073 1,066		1,024 1,251 1,274
4	5.5	4,120 3,600 3,034 4,000 3,600 3,437	1,036 1,137 1,260 1,160		1,274
6	28	8,800 6,000 7,400	1,285 1,320		865 1,019 1,061 1,134
7				125 135	
8	86	13,000 10,860 12,657	1,373 1,400 1,420		
9				135 122	1,349 1,221 1,040 1,279
10	145	21,200 16,300 21,430	1,486 1,460 1,500		
12				146 109	1,307 653 920

Résumé

Polysaccharide bakterieller Herkunft

On démontre que les acides aminés libres du vitellus liquéfié augmentent pendant le développement de l'embryon, ce qui suggère une hydrolyse de ses protéines.

Wirkung von Polysacchariden bakterieller Herkunft auf die experimentelle Lipämie

In früheren Arbeiten unseres Laboratoriums wurden verschiedene pharmakologische Wirkungsqualitäten von Polysacchariden bestimmter Art beschrieben, wie die spezifische Wirkung auf die Chemotaxis der Leukozyten¹, auf die entzündlichen Reaktionen des Bindegewebes², sowie auf verschiedene Infektions-³ und anaphylaktische⁴ Phänomene.

Zu den bekannten Wirkungsqualitäten bestimmter Polysaccharide, wie zum Beispiel Heparin und Manuronat, gehört auch deren Antilipämie- und blutgerinnungshemmende Wirkung. Es schien deshalb wichtig, Polysaccharide verschiedener Art und Herkunft auch auf ihre antilipämische Wirkung *in vivo* und auf ihre Wirksamkeit auf die Plasmagerinnung *in vitro* zu untersuchen.

Die *Antilipämie-Wirkung in vivo* wurde, wie üblich, an Hand der « Klärung » des Serums lipämischer Ratten bestimmt, im wesentlichen nach der von CONSTANTINIDES *et al.* angegebenen Methode⁵. Eine Gruppe von mindestens 5 bis 10 Ratten erhält nach 16stündigem Hungern je Ratte 3 ml Maisöl pro 100 g *per os* (enthaltend 0,3 g Zellulose, um Intoleranzerscheinungen zu vermeiden) und bleibt unbehandelt. Sie dient als Kontrollgruppe. Die 5–10 Ratten jeder anderen Gruppe erhalten unmittelbar nach Maisölverabreichung je die gleiche Dosis des zu prüfenden Präparates intravenös in die Schwanzvene. 2 h nach der Injektion werden die Tiere in Avertinarkose (1 ml/100 g intraperitoneal einer 2prozentigen Lösung) aus der Carotis in ein Zentrifugenglas entblutet, das Blut 30 min bei 3000 U./min zentrifugiert, das Serum abgehoben, 1:5 mit Wasser verdünnt und der Trübungswert einzeln bestimmt. Als Kontrolle dient das gesammelte 1:5 verdünnte Serum der Kontrollratten (1 ml Serum jedes Tieres). Die Trübung wird je Tier nephelometrisch in einem Coleman-Spektrophotometer (Mod. 14) bei 610 mμ in Prozent Transmission abgelesen, nachdem das verdünnte Mischserum der Kontrollratten auf 100% Transmission gestellt worden ist. Das arithmetische Mittel der Trübungswerte der Ratten je Präparatdosis (im Vergleich zum Kontrolltrübungswert = 100%) gibt die *prozentuale Klärung* durch die injizierte Präparatdosis. Die Präparatdosen werden so gewählt, dass eine 50prozentige Klärung durch mehrere Dosen (pro Gruppe) sowohl unter- wie überschritten wird, so dass durch Interpolation die Dosis eruiert werden kann, welche 50prozentige Klärung verursacht (= ED₅₀). Die *blutgerinnungshemmende Wirkung* wurde im Rekalzifizierungsversuch an Rinderoxalatplasma *in vitro* bei 37°C untersucht.

Befunde. Die in der Tabelle zusammengestellten Präparate umfassen nur einen Teil der von uns geprüften, und zwar nur die antilipämisch wirksamsten Präparate. Die Einteilung erfolgte nach deren Herkunft, Fraktionierung und Antilipämiewirkung. Die Wirksamkeit ist ausgedrückt in Effektivdosen.

¹ R. MEIER, Z. exp. Med. 87, 283 (1933). – R. MEIER und B. SCHÄR, Exper. 9, 93 (1953); 10, 376 (1954); Z. physiol. Chem. (im Druck).
² R. MEIER, P. A. DESAULLES und B. SCHÄR, Arch. exp. Path. Pharm. 224, 104 (1955).
³ R. MEIER und L. NEIPP, Schw. med. Wschr. 86, 249 (1956).
⁴ R. MEIER, H. J. BEIN und R. JAQUES, Exper. 12, 235 (1956).
⁵ P. CONSTANTINIDES, A. CAIRNS und A. WERNER, Canad. Res. Conc. Abstr. West. Reg. Meet. 19, 1954.

Charakterisierung der Präparate	Antilipämie ED ₅₀ % Klärung γ/kg intraven.	Rekalzifizierung (Gerinnung <i>in vitro</i>)	
		E. K. %	Wirkung
1. Aus gramnegativen Bakterien (78 Präparate untersucht)			
A = Äthanol-fällung aus Kulturfiltrat	1000		
Subfraktionierung von A:			
Aa	4	0,05	Ø
Ab	20		
Ac	35		
Ad	35		
Ae	60		
Af	70		
Ag	100		
Ah	1000		
Ai	1000		
Subfraktionierung von Aa:			
Aa/1.	8	0,05	Ø
Aa/2.	10		
Aa/3.	1000		
B = Mit 3 Volumen Äthanol aus Kulturfiltrat nicht fällbar	10000		
Subfraktionierung von B:			
Ba	250		
Bb	500		
Bc	1000		
C = Fraktionierung des Kulturfiltrats unter Vermeidung organischer Lösungsmittel	400	0,1	Ø
D = Fraktion aus Bakterienrückstand	75		
Subfraktionierung von D:			
Da	1	0,05	Ø
Db	4		
E = Kombination der Fraktionierung nach D, Da, Db aus Bakterienrückstand	1	0,05	Ø
F = Nebenfraktion von E . . .	5	0,05	Ø
G = Nebenfraktion von F. . .	1000	0,05	Ø
2. Aus grampositiven Bakterien (2 Präparate untersucht) . . .	0	0	
3. Aus Mykobakterien (6 Präparate untersucht) . . .	0	0	

Die *Polysaccharide aus gramnegativen Bakterien* wurden aus dem Kulturmedium oder auch aus Bakterienrückständen von Proteus gewonnen und auf verschiedene Weise fraktioniert⁶. Die Beziehung der einzelnen Präparate zueinander hinsichtlich ihrer Fraktionierung ist aus der Tabelle ersichtlich. Die in der Tabelle angegebenen wirksamsten aller als wirksam zu bezeichnenden Präparate dürften in der Hauptsache den Lipopolysaccharidfraktionen zuzurechnen sein.

Unter diesen Präparaten finden sich Fraktionen, welche in Effektivdosen ab 1 γ/kg intravenös die experimentelle Lipämie an Ratten um 50% vermindern. Es gelingt somit die bei der Lipämieklärung *in vivo* wirksame Substanz anzureichern. Angegeben sind nur solche Präparate, deren Effektivdosis 1 mg/kg intravenös

⁶ Die Präparate bzw. Fraktionen wurden in unseren chemischen Laboratorien von Dr. F. W. KAHNT dargestellt.

nicht überschreitet; letztere sind etwa so wirksam wie Heparin (Hoffmann-La Roche) mit einer $ED_{50} = 3,2$ mg/kg intravenös. Keines dieser Präparate erwies sich, soweit geprüft, als blutgerinnungshemmend wirksam.

Der Wirkungsmechanismus der Klärung ist von demjenigen des Heparins verschieden, er wird noch untersucht und später im einzelnen dargelegt werden.

Zwei Präparate von Polysacchariden aus grampositiven Bakterien und sechs von Polysacchariden aus Mykobakterien erwiesen sich als antilipämisch und hinsichtlich Plasmagerinnung als unwirksam.

Polysaccharidpräparate pflanzlicher und tierischer Herkunft, von denen eine Auswahl geprüft wurde, erwiesen sich antilipämisch als wesentlich weniger wirksam als die beschriebenen Bakterienpolysaccharide.

R. MEIER und W. SCHULER

Forschungslaboratorien der CIBA Aktiengesellschaft Basel, Pharmazeutische Abteilung, den 5. April 1957.

Summary

Polysaccharide fractions derived from a gram-negative proteus organism show strong anti-lipæmic activity of the order of 1000 times that of heparin. Analogous preparations from gram-positive organisms and mycobacteria show no such activity and some polysaccharide fractions from plant and animal tissues proved to have slight activity. None of these polysaccharides influenced the clotting of plasma *in vitro*.

Lack of Effect of Exercise on Serum Cholesterol Levels in two Types of Experimental Obesity¹

Considerable interest has developed recently about the possibility that the lack of physical activity, which is a factor in obesity² may also be a factor predisposing to atherosclerosis and thus coronary heart disease³. Inasmuch as a high serum cholesterol level in certain species appears to be an important factor in the development of atherosclerosis³, it appeared of interest to examine the effect of exercise on the serum cholesterol levels of normal mice and of mice with experimental obesity. The two types chosen are representative of the two general classes of obesity. Goldthiogluucose obese mice, where the primary lesion is the destruction of specialized cells in the ventromedial hypothalamic area of the hypothalamus⁴, is representative of the 'regulatory' forms of obesity², where metabolic abnormalities are secondary to hyperphagia. Hereditarily obese-

hyperglycemic mice present a syndrome, characterized by a variety of endocrine, enzymatic and metabolic idiosyncrasies⁵—in particular hypercholesterolemia⁶. They are representative of the 'metabolic' forms of obesity where hyperphagia appears to be secondary to metabolic abnormalities.

A total of 53 mice were used in the study. Goldthiogluucose obese mice were obtained as previously described⁷ by injection of an LD_{50} dose of goldthiogluucose 3 months previously and weighed 40 to 70 g. Mice with the genetic syndrome had been bred at the Jackson Memorial Laboratory, Bar Harbor, Maine; they were from 5 to 8 months old and weighed 40 to 60 g. The exercised animals were put on the treadmill previously described⁸ and exercised as in this previous experiment, the total duration of actual exercise excluding pauses was 1 h a day at a speed of 0.435 Km/h. All animals were weighed daily. The obese animals had been steadily gaining weight until the beginning of the exercise period. The experimental period lasted 30 days. During the experimental period and in conformity with previous results², exercised obese mice had gained less than their controls, on the average 5 g less in the case of the obese-hyperglycemic mice and 12 g less in the case of the goldthiogluucose animals. These weight differences were highly significant ($p < 0.01$). Non-obese mice had the same weight in the exercised and non-exercised group.

Serum cholesterol levels were determined by the KENDALL-ABELL method⁹. Results are given in the Table. None of the differences in serum cholesterol between exercised and non-exercised animals are significant. The experiment presented here thus does not support the idea that exercise is a major factor in determining cholesterol levels. It is of course conceivable that had the experiment been continued beyond a month, or had the exercise been more severe, a difference might have appeared. It should also be pointed out that the existence of a species difference is entirely possible and even likely. We have not detected atherosclerosis in mice including the hereditarily obese mice with hypercholesterolemia. However, should these results relate to the problem of human atherosclerosis they might be taken as suggesting the possibility that favorable results of physical activity may be mediated through an effect other than variations of cholesterol levels.

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Department of Nutrition, Harvard School of Public Health, Boston, Mass., February 12, 1957.

Résumé

Divers auteurs ont supposé récemment que la pratique journalière d'exercices physiques peut abaisser le taux du cholestérol du sang. Cette hypothèse a été testée chez des souris normales et chez des souris représentant deux classes d'obésité: des souris porteuses du syndrome héréditaire d'obésité-hyperglycémie (qui est accom-

¹ This work was supported in part by grants from the National Institute of Arthritis and Metabolic Diseases (grant No. A-49), National Institutes of Health, Public Health Service, Bethesda; and the Fund for Research and Teaching, Department of Public Health, Harvard School of Public Health, Boston.

² J. MAYER, Nutr. Abstr. Rev., Part I 25, 597 (1955); Part II 25 871 (1955).

³ J. N. MORRIS and J. A. HEADY, Brit. J. ind. Med. 10, 245 (1953). — J. N. MORRIS, J. A. HEADY, P. A. B. RAFFLE, C. G. ROBERTS, and J. W. PARK, Lancet 1953, 1053 and 1111. — A. KEYS *et al.* in Arteriosclerosis, A Symposium (Univ. of Minn. 1956), *Mode of life*, A. KEYS, p. 28; *Cholesterol Metabolism*: I. D. FRANTZ, jr., p. 49.

⁴ N. B. MARSHALL, R. J. BARNETT, and J. MAYER, Proc. Soc. exp. Biol. Med. 90, 240 (1955).

⁵ J. MAYER, Physiol. Rev. 33, 472 (1953). — K. H. SHULL, J. ASHMORE, and J. MAYER, Arch. Biochem. Biophys. 62, 210 (1956). — K. H. SHULL and J. MAYER, J. biol. Chem. 218, 885 (1956).

⁶ J. MAYER and A. K. JONES, Amer. J. Physiol. 175, 339 (1953).

⁷ N. B. MARSHALL and J. MAYER, Amer. J. Physiol. 178, 271 (1954).

⁸ J. MAYER *et al.*, Amer. J. Physiol. 177, 544 (1954).

⁹ L. L. ABELL, B. B. LEVY, B. B. BRODIE, and F. E. KENDALL, J. biol. Chem. 195, 357 (1952).

Effect of exercise on serum cholesterol levels. In normal mice and in mice with two experimental obesities

Type	Number of Animals	Activity	Average WT at start g	Average WT -at end g	Serum Cholesterol mg/%
Obese Hyperglycemic	6	non-exercised	55 ± 8	57 ± 6	170 ± 24
	6	exercised	55 ± 11	53 ± 9	192 ± 24
Nonobese Littermates of Obese-Hyperglycemic Mice	6	non-exercised	27 ± 6	28 ± 7	116 ± 10
	6	exercised	26 ± 7	26 ± 5	98 ± 39
Goldthiogluucose Obese Swiss Mice	9	non-exercised	53 ± 5	57 ± 8	125 ± 24
	6	exercised	51 ± 7	43 ± 9	123 ± 17
Uninjected Swiss Mice	8	non-exercised	28 ± 4	28 ± 3	112 ± 41
	6	exercised	29 ± 3	31 ± 5	96 ± 13

pagnée d'une hypercholestérimie génétique), représentant l'obésité «de métabolisme», et des souris rendues obèses par les lésions hypothalamiques causées par l'administration d'aurothiogluucose, représentant l'obésité «de régulation». Trente jours d'exercice (une heure de course sur tapis roulant par jour) n'eurent aucun effet sur le taux du cholestérol du serum ni chez les souris normales, ni chez les deux groupes de souris obèses, et ceci en dépit du fait que la durée et l'intensité de l'exercice quotidien étaient suffisantes pour réduire de façon significative le poids des souris obèses.

Über den Vitamin-B₁₂-Gehalt von Organen verschieden ernährter Ratten

Die zahlreichen tierexperimentellen Untersuchungen über die Wirkung von Vitamin B₁₂ haben oft einander widersprechende Resultate ergeben. Dies dürfte zum Teil darauf zurückzuführen sein, dass von den einzelnen Forschern sehr ungleiche Fütterungsmethoden angewendet werden und deshalb der erzielte Mangelzustand mehr oder weniger ausgeprägt ist. Dieser lässt sich am besten charakterisieren durch Bestimmung der im Tier verbliebenen Vitaminreserven. Im folgenden berichten wir über Resultate von Untersuchungen des Vitamin-B₁₂-Gehaltes in Niere und Leber bei Ratten nach verschiedenen diätischen Behandlungen.

Männliche. 4–6 Monate alte Albinratten des «Sprague-Dawley»-Stammes wurden benützt. Die Tiere der Versuchsgruppen waren über mehr als zehn Generationen auf den entsprechenden Diäten gezüchtet worden. Die Kontrolltiere erhielten ein kommerzielles Rattenfutter mit einem Vitamin-B₁₂-Gehalt von etwa 3 µg/100 g. Die B₁₂-Mangeldiät war folgendermassen zusammengesetzt¹: entfettetes Soyamehl 46, Maismehl 46, Salzmischung 2, Sesamöl mit Vitaminen A, D und E 5, Vitamin-B-Mischung (ohne B₁₂) 1. Die Vitamin-B₁₂-Bestimmungen wurden mit *L. leishmannii* nach einer Modifikation der in U.S. Pharmacopeia XIV angegebenen Methode ausgeführt. In einigen Fällen wurden ebenfalls die alkaliresistenten Blindwerte bestimmt, die aber stets so niedrig waren, dass sie vernachlässigt werden konnten. Die Tiere wurden gewogen, durch Schlag getötet, Leber und Nieren entnommen, gewogen und rasch in Phosphat-Zitrat-Puffer von pH 4,5 homogenisiert und

in Gegenwart von Bisulfit während 15 min im Autoklaven erhitzt.

Die Ergebnisse sind in der Tabelle zusammengefasst. In den Nieren war der Vitamin-B₁₂-Gehalt stets höher als in der Leber, in beiden Organen waren die relativen Schwankungen immer ungefähr gleich gross. Die Resultate änderten sich nicht merklich, wenn statt der absoluten Konzentration des Vitamins der relative B₁₂-Gehalt der Organe in Betracht gezogen wurde.

Nach Mais-Soyadiät wurde im Vergleich zu den Kontrollen nur etwa 1/10 der Vitaminmenge gespeichert. Wurden der Diät 5 µg/kg B₁₂ zugefügt, was normales Wachstum und Fortpflanzung erlaubt, war der Gehalt in den Organen etwa doppelt so hoch. Nach 3 Wochen Mais-Soyadiät war die B₁₂-Konzentration der Organe auf die Hälfte vermindert (Serie 8); wurde zu dieser Diät aber 0,1% jodiertes Kasein gegeben, so war nach 3 Wochen eine Abnahme des B₁₂-Gehaltes auf etwa 1/3 festzustellen (Serie 6). Nach Verabreichung der Kontrolldiät oder der Soyadiät mit einem Zusatz von 30 µg/kg B₁₂ an Mangeltiere während eines Monats waren die B₁₂-Reserven in den untersuchten Organen normal (Serien 9 und 10).

Verfütterung von Mais-Soyadiät mit jodiertem Kasein an Mangeltiere während 3 Monaten ergab keine wesentliche Abnahme des Vitamin-B₁₂-Gehaltes (Serie 7). Bei Mangeltieren werden also die festgestellten Vitaminmengen viel stärker zurückgehalten als bei Kontrollen. Zur Erklärung könnte angenommen werden, dass in den Organen der Mangeltiere das Vitamin B₁₂ mehr oder weniger vollständig an Fermente gebunden ist, während bei normalen Tieren ein wesentlicher Teil als mobilisierbare Reserve vorhanden ist.

Aus den angeführten Daten geht hervor, dass auch Tiere, die über mehr als 10 Generationen mit B₁₂-freier Diät ernährt worden waren, stets geringe, wohl durch die Darmflora synthetisierte Mengen dieses Vitamins enthalten. Ausserdem zeigt sich, dass eine dreiwöchige Fütterung mit B₁₂-Mangeldiät nicht ausreicht, um die Vitaminreserven aufzubrauchen, selbst dann nicht, wenn die Nahrung jodiertes Kasein enthält.

Die bei Kontroll- und Mangeltieren gefundenen Vitamin-B₁₂-Werte stimmen mit den Angaben anderer Autoren gut überein².

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Nationales Ernährungsinstitut, Caracas (Venezuela), den 17. Januar 1957.

¹ W. G. JAFFÉ, J. Nutr. 59, 135 (1956).

² M. MOINUDDIN und O. G. BENTLEY, J. Nutr. 56, 335 (1955).

Vitamin B₁₂ in Leber und Nieren von verschieden ernährten Ratten

Nr.	Anzahl von Tieren	Versuchsdiät	Versuchsdauer	Vorherige Diät	Vitamin B ₁₂ in µg/g		Relativer Vitamin-gehalt ³
					Lebern	Nieren	
1	9	Kontrollen	von Geburt an	—	0,204 ± 0,016 ⁴	1,421 ± 0,032	1,95
2	8	Soya-Mais	von Geburt an	—	0,022 ± 0,002	0,106 ± 0,005	0,156
3	8	Soya-Mais + 3 µg/kg Vit. B ₁₂	von Geburt an	—	0,040 ± 0,004	0,182 ± 0,022	0,252
4	4	Soya-Mais + 0,2% jodisiertes Kasein	1 Woche	Kontrollen	0,130 ± 0,036	1,057 ± 0,150	1,56
5	4	Soya-Mais + 0,1% jodisiertes Kasein	2 Wochen	Kontrollen	0,0906 ± 0,017	0,304 ± 0,027	0,716
6	4	Soya-Mais + 0,1% jodisiertes Kasein	3 Wochen	Kontrollen	0,0760 ± 0,008	0,269 ± 0,043	0,645
7	4	Soya-Mais + 0,1% jodisiertes Kasein	12 Wochen	Soya-Mais	0,015 ± 0,001	0,092 ± 0,006	0,177
8	4	Soya-Mais	3 Wochen	Kontrollen	0,127 ± 0,018	0,488 ± 0,009	0,840
9	4	Kontrollen	4 Wochen	Soya-Mais	0,177 ± 0,022	1,129 ± 0,131	1,61
10	6	Soya-Mais + 30 µg/kg Vit. B ₁₂	4 Wochen	Soya-Mais	0,197 ± 0,029	1,594 ± 0,069	1,96

³ $\frac{\text{Gesamtmenge von B}_{12} \text{ in Leber + Nieren}}{\text{Körpergewicht}} \times 100$.

⁴ Standardfehler des Mittelwertes.

Summary

Adult, male rats bred for over 10 generations on a soy meal-corn-diet had vitamin B₁₂ values of liver and kidney about 10 times lower than the controls. If the deficient ration was supplemented with 5 µg/kg of B₁₂, these values were still about 1/5 of the controls. 3 weeks on the deficient diet lowered the B₁₂ levels in the livers and kidneys of previously undepleted rats to about 1/2, and a similar diet containing 0.1% of iodized casein lowered these levels to about 1/3 of the normal values but did not lower the B₁₂-concentration of organs of already deficient rats.

Rats bred on the deficient diet and receiving for 1 month a supplement of 30 µg/kg of vitamin B₁₂ or the stock diet with a similar B₁₂-content, had normal B₁₂-levels in livers and kidneys.

Urethane: ethyl carbamate

Myleran (Mielucin): butyl-1, 4-di(methylsulphonate) 6-Mercaptopurine.

Susceptibility measurements were carried out soon after preparation of the substances, because of the lability of the latter. These values were obtained by working with a magnetometric balance of Weiss-Föex type, modified by MAYR². Measurements were related to triply distilled water whose susceptibility (0.72×10^{-6}) had been previously controlled by comparative measurements with doubly distilled mercury. Temperature 20–22°C.

The susceptibility calculations were carried out according to the magnetochemical systematology of PACAULT³ based on PASCAL's experimental data. The values calculated in this way are based on the contribution to diamagnetism of single atoms (atomic susceptibility) and bonds between atoms of the same molecule (constitutive increments). These values are, in many cases so far considered, in agreement with the experimental results within a limit of 2%. Discordant values occur when the constitutive increments are not yet known with sufficient precision; in these cases the calculated values must be considered as indicative data and such discordance may be a useful indication for the detection of constitutional characteristics still unknown.

Calculated and experimental data are tabulated.

They show, as a general characteristic, that the experimental values are superior to the calculated ones; the hypothesis is therefore advanced that in these substances one or more π -electrons are spread on the adjacent atoms. In a later paper, one of us (MAYR) will discuss from the theoretical standpoint the admissibility of this assumption and the possibility of a relationship between particularly extended orbits and corresponding regions of greater electronic rarefaction, occasionally coexisting, however, with centers of higher electronic density.

Without venturing to explain a phenomenon which is still rather complex, it may be thought that the advanc-

Magnetochemical Behaviour of some Substances Used in the Medical Treatment of Tumors

In a previous research¹, the magnetochemical behaviour of some carcinogenic substances was examined.

We now considered that the study of the magnetic susceptibility of some chemo-therapeutical substances, of interest for their antineoplastic action, which cannot at present be considered from the standpoint of theoretical chemistry, might be profitable to investigate (at least approximately) in view of the possible presence and type of electronic anomalies.

The following substances were examined:

DMC: di-(2-chloroethyl)-methylamine hydrochloride
 Chloronaphthine: di-(2-chloroethyl)- β -naphthylamine
 TEM: 2, 4, 6-triethylenimine-1, 3, 5-triazine
 PEI: ethyleneimine picrate
 EMU: N, N'-dicycloethylenecarbamylhexamethylene-diamine

¹ P. RONDONI, G. MAYR, and E. GALICO, *Exper.* 5, 357 (1949).

² G. MAYR, *R. C. Ist. Lombardo Sci. Lett.* 83, 3 (1950).

³ A. PACAULT, *Rev. Sci.* 86, no. 3288, p. 38 (1948).

Summary of Results

Substance	Mol. weight	$\chi \cdot 10^6$		$\chi_{\text{mol}} \cdot 10^6$		Difference 10 ⁶	Number of de- termi- nation **
		calculated	observed	calculated*	observed		
DCM	192,5	−0.68	−0.81 ± 0.01	−131	−155.9 ± 2	25	12
Chloronaphthine	268	−0.68	−0.74 ± 0.02	−181.8	−198.3 ± 5.4	16	8
EMU	254	−0.61	−0.82 ± 0.02	−152.2	−208.3 ± 5.1	56	12
Mercaptopurine	152	−0.46	−0.64 ± 0.02	−69.7	−97.3 ± 3	27	8
Myleran.	246	−0.55	−0.69 ± 0.02	−135.6	−169.7 ± 4.9	34	8
PEI***	272	−0.40	−0.58 ± 0.02	−110	−157.8 ± 5.4	48	8
TEM	204	−0.61	−0.74 ± 0.02	−123.8	−150.9 ± 4	27	6
Urethane	89	−0.45	−0.64 ± 0.02	−40.4	−56.9 ± 1.8	16	10

* The calculated values concern only the contributions given to the susceptibility by atoms and constitutive increments at present known.

** The mean value of more consecutive measurements (usually 6) was calculated as a single determination.

*** Picric acid, for which 3 measurements were carried out, showed the value 20 as difference between calculated and observed susceptibility, parallel to what is observed for PEI. This fact shows that the anomaly observed for PEI is dependent in part on the trinitrophenol ring.

ed hypothesis, concerning the particularly extended electronic orbits could perhaps explain the antineoplastic mechanism of the above mentioned substances, if considered analogous to the interaction between inhibitory and carcinogenic substances⁴. Further, the possible relationship between the extended electronic orbits and the occasional recurring of corresponding regions of higher electronic density, might perhaps also explain the carcinogenic activity of most of the compounds examined.

In conclusion, even considering the anomalies mentioned only from a presumptive point of view, the substances showing antineoplastic activity—as well as those known as carcinogenic—are characterized by anomalies of the electronic cloud inherent with their molecules.

G. MAYR and G. C. RABOTTI

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Sommario

Fu misurata la suscettività diamagnetica di alcune sostanze usate nella chemioterapia dei tumori con lo scopo di confrontare i risultati così ottenuti coi valori della stessa calcolati in base alle sistematiche magnetochimiche. Nei limiti di precisione consentiti dalle conoscenze attuali di queste sistematiche, si è potuto notare una divergenza fra valori osservati e calcolati tale da indurre ad ammettere l'esistenza di anomalie strutturali, precisamente di irregolarità della nube elettronica inerente alle molecole delle sostanze esaminate.

⁴ H. C. CRABTREE, *Cancer Research* 5, 351 (1945).

The Effect of Thyroid Function on the Prothrombin Time Response to Warfarin in Rats*

The coagulation of blood has been reported to be increased in hypothyroid¹ and decreased in hyperthyroid²

* This work was supported by a grant from the National Research Council of Canada.

¹ K. KOTTMANN, *Z. klin. Med.* 71, 361 (1910).

² R. GORDON and B. A. LAMBER, *Acta endocrin.* 19, 77 (1955).

conditions. Although the changes from normal are small, these observations indicate that the level of thyroid function has some effect on the concentration of various coagulation factors. Since indirect anticoagulants of the dicoumarol type act by depressing the synthesis of such factors, their activity in the hypo- and hyperthyroid rat has been investigated. Warfarin (3-[⁹]- α -phenyl- β -acetyylethyl[⁸]-4-hydroxycoumarin), a member of this group of anticoagulants, was used because in the rat it causes a greater and more consistent change of the prothrombin time than dicoumarol.

Male rats, weighing from 175 to 225 g were made hypothyroid by the daily administration in the drinking water of 2 mg of Tapazole (1-methyl-2-mercapto-imidazole)³ per day, for 8 weeks, and hyperthyroid by the subcutaneous injection of 200 μ g of *l*-thyroxin on 4 alternate days. Warfarin⁴, at a dose of 500 μ g per 100 g of body weight per day was given orally mixed with the feed. Blood samples were taken by tail vein puncture from unanesthetized animals and the prothrombin time determined on whole blood by the method of SCHWAGER and JAKUES⁵.

In the hyperthyroid group a statistically significant prolongation of the prothrombin time was found before anticoagulant treatment. The effect of the anticoagulant on the prothrombin time was further significantly increased in the hyperthyroid and significantly decreased in the hypothyroid animals (Table).

The results suggest that in the hypo- and hyperthyroid state the rate of synthesis of factors regulating the prothrombin time is at a level different from normal even if the prothrombin time shows little change. However, the deviation from normal is magnified by the administration of the anticoagulant.

The state of thyroid function has been shown to have an influence on many physiological functions, often in an indirect manner. For example, the increased sympathetic tone or response to adrenaline in the hyperthyroid animal has been stated to be due to a decreased level of amine oxidase, which results in a decreased rate of inactivation of the sympathetic neurohumoral transmitter or administered adrenaline⁶.

³ Kindly supplied by Eli Lilly and Co., Indianapolis, USA.

⁴ Kindly supplied by S. B. Penick and Co., New York, USA.

⁵ P. G. SCHWAGER and L. B. JAKUES, *Canad. med. Assoc. J.* 60, 258 (1949).

⁶ H. SPINKS, *J. Physiol.* 117, 35 P (1952).

Effect of Thyroid Function on Prothrombin Time Response to Warfarin in Rats.

Treatment	Number of Animals	Before Anticoagulant, Prothrombin time, s ± S.E.	First Day on Anticoagulant, Prothrombin time, s ± S.E.	Second Day on Anticoagulant, Prothrombin time, s ± S.E.	Third Day on Anticoagulant, Prothrombin time, s ± S.E.
Normal	10	28.8 ± 1.0	100.0 ± 13.7	190.0 ± 14.5	
Hyperthyroid . . .	10	34.5 ± 1.6*	163.1 ± 17.4*	251.0 ± 19.5**	
Normal	10	31.6 ± 1.3	107.9 ± 7.2	241.1 ± 4.8	424.0 ± 50.0
Hypothyroid . . .	10	34.6 ± 0.7	79.1 ± 6.8*	225.0 ± 7.3	245.0 ± 24.6*

* Difference between means of normal and experimental groups are significant at a probability level < 0.01.

** Difference between means of normal and experimental groups are significant at a probability level < 0.05.

The decreased response to the anticoagulant in the hypothyroid state may likewise be due to an increased rate of synthesis or more probably a decreased rate of degradation of prothrombin and other coagulation factors at the cellular level, while the increased metabolic activity in the hyperthyroid state would tend to increase the effect of the anticoagulant by accelerating protein degradation.

Recently, MARTIUS *et al.*⁷ have postulated that indirect anticoagulants like dicoumarol may act in a manner similar to thyroxine. Both dicoumarol and thyroxine decrease, while Vitamin K increases, oxidative phosphorylation of mitochondria *in vitro*. The inhibitory activity of coumarin derivatives was found to parallel anticoagulant activity. Dicoumarol has also been shown to inhibit DPNH oxidase activity⁸.

These observations are difficult to correlate with the anticoagulant activity of these drugs in the intact animal, where their action is highly specific. Doses which cause a significant decrease of prothrombin levels fail to alter liver functions or change the levels of liver enzymes⁹, or cause any other widespread physiological changes one would associate with an uncoupling of oxidative phosphorylation. Hence, while the results demonstrate a synergism between thyroid hormone and indirect anticoagulants, their site or mode of action at the cellular level may not necessarily be identical.

J. LOWENTHAL and L. M. FISHER

Department of Physiology, University of Saskatchewan, Saskatoon (Canada), January 30, 1957.

Zusammenfassung

Die Wirkung von Warfarin, einem Anticoagulans der Dicoumarolgruppe, ist erhöht bei hyperthyroiden und vermindert bei hypothyroiden Ratten.

⁷ C. MARTIUS, *Proceedings of the 3rd International Congress of Biochemistry*, Brussels 1956 (Academic Press Inc., New York 1956).

⁸ J. LOWENTHAL, *Bull. Soc. chim. Belg.* 65, 124 (1956).

⁹ J. P. GREEN, E. SØNDERGAARD, and H. DAM, *Acta pharmacol. et toxicol.* 11, 79 (1955).

The Influence of Inorganic Thiophosphate on the Conversion of Parathion to Paraoxon in Rat Liver Slices

As was shown by various authors¹ parathion is converted in the animal body to its oxygen analogue, paraoxon, which is a powerful anticholinesterase; therefore, this metabolic conversion is of primary importance in the toxic action of parathion.

The identity of the enzymic system involved is not clear; the corresponding dehydrogenase seems to be DPN-linked². GAGE³ suggested that the system may be identical with that of BINKLEY⁴ which catalyses the oxidation of inorganic thiophosphate to orthophosphate and perhaps thiosulphate. If this were true, inorganic thiophosphate when present in a sufficiently high concentration should exhibit an inhibitory influence on the conversion of parathion to paraoxon with this system. Examples of such competition of substrates are known even from *in vivo* experiments, e.g. the oxidation of methyl alcohol was found to be retarded by doses of ethyl alcohol⁵ etc.

Therefore, the rate of paraoxon formation was followed in female rat liver slices incubated with various concentrations of parathion in the presence of various concentrations of sodium thiophosphate (Na₂HPSO₃·12 H₂O). The concentrations of the later substance were held within the range that would be innocuous to the experimental animal when injected (the intravenous LD₅₀ of the preparation used was 432 mg/kg for female mice and approximately 800 mg/kg for female rats). The incubation was continued until a steady state was reached in which paraoxon was formed as rapidly as it was split by the A-esterase present⁶, so that its concentration in the medium did not change. This steady state concentration, which was reached after 60–90 min in the conditions of the experiment, is a measure of the velocity of paraoxon formation, if the rate of this decomposition is supposed to be constant⁷. To be sure that the con-

¹ D. K. MYERS, B. MENDEL, H. R. GERSHMANN, and J. A. A. KETELAAR, *Nature* 170, 815 (1952). – R. L. METCALF and R. B. MARCH, *Ann. entomol. Soc. Amer.* 46, 63 (1953). – J. C. GAGE, *Biochem J.* 54, 426 (1953).

² A. N. DAVISON, *Biochem. J.* 61, 203 (1955).

³ J. C. GAGE, *Biochem. J.* 54, 426 (1953).

⁴ F. BINKLEY, *J. biol. Chem.* 181, 317 (1949).

⁵ L. P. KENDAL and A. N. RAMANATHAN, *Biochem. J.* 54, 425 (1953).

⁶ W. N. ALDRIDGE, *Biochem. J.* 53, 117 (1953).

⁷ J. KUBIŠTOVÁ, *Exper.* 12, 233 (1956).

centration of thiophosphate inside the tissue was constant from the beginning of the experiment, the slices were pre-incubated with thiophosphate before parathion was added to them. Controls with the slices of the same animal without thiophosphate were run simultaneously and the results corrected according to them.

In these conditions, inorganic thiophosphate was found to have a distinct inhibitory influence on the enzymic formation of paraoxon from parathion. The inhibition, however, did not decrease with rising concentration of the substrate, i.e. parathion, if the concentration of the inhibitor, i.e. thiophosphate, was kept constant, as would be expected in the case of the competitive inhibition. On the contrary, the inhibition increased with increasing substrate concentration, as may be seen from the following table:

Parathion concentration	Thiophosphate concentration	Paraoxon steady state concentration	Inhibition in %
$5 \cdot 9 \times 10^{-5} M$	0	$6 \cdot 1 \times 10^{-7} M$	0
$5 \cdot 9 \times 10^{-5} M$	$9 \cdot 44 \times 10^{-3} M$	$3 \cdot 0 \times 10^{-7} M$	49
$1 \cdot 18 \times 10^{-4} M$	0	$8 \cdot 2 \times 10^{-7} M$	0
$1 \cdot 18 \times 10^{-4} M$	$9 \cdot 44 \times 10^{-3} M$	$2 \cdot 2 \times 10^{-7} M$	73
$3 \cdot 95 \times 10^{-4} M$	0	$2 \cdot 6 \times 10^{-6} M$	0
$3 \cdot 95 \times 10^{-4} M$	$9 \cdot 44 \times 10^{-3} M$	$3 \cdot 1 \times 10^{-7} M$	88

If the values found are plotted into a diagram, it appears that the relation of the reciprocal of the reaction velocity, substrate (parathion) concentration and inhibitor (thiophosphate) concentration fit reasonably well with the law of anticompetitive inhibition (Fig. 1 and 2)⁸. It seems that this relation is not the result of some non-specific action of thiophosphate, e.g. the suppression of the general metabolism of the cells; it was found in accordance with BINKLEY⁴ that thiophosphate is oxidized by liver slices. In the conditions of the experiments, 20–30% of the original thiophosphate present was found to be oxidized at the end of the experiment.

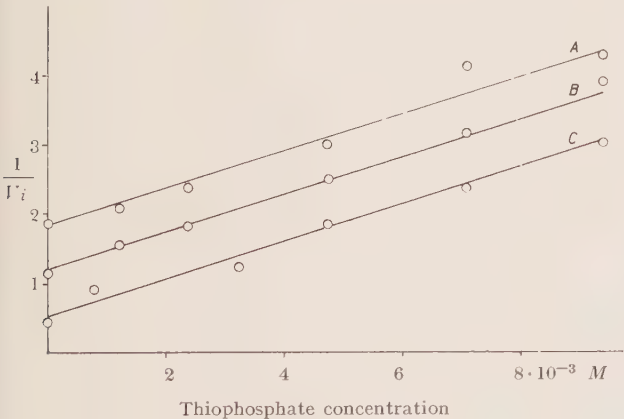


Fig. 1.—Relation between $1/V_i$ and $[I]$ using 3 different concentrations of parathion: A $5 \cdot 9 \times 10^{-5} M$, B $1 \cdot 18 \times 10^{-4} M$, C $3 \cdot 95 \times 10^{-4} M$.

The enhancement of oxygen consumption corresponded well with the oxidation of the sulphur moiety of the

molecule to the thiosulphate level, as supposed by BINKLEY⁴.

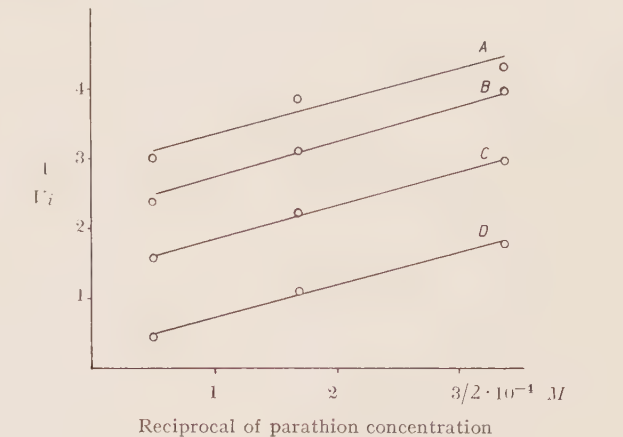


Fig. 2.—Relation between $1/V_i$ and $1/[S]$ using four different concentrations of thiophosphate: A $9 \cdot 44 \times 10^{-3} M$, B $7 \cdot 08 \times 10^{-3} M$, C $4 \cdot 72 \times 10^{-3} M$ and D 0.

Therefore, it seems possible that the above relation reflects the interaction of the substrate and inhibitor with the enzyme responsible for the parathion to paraoxon conversion. The anticompetitive type of inhibition, which was found in this case and which suggests the reaction of the inhibitor with the enzyme-substrate complex does not, however, justify the conclusion that both inorganic thiophosphate and parathion are metabolised by the same enzyme system.

J. KUBIŠTOVÁ

Institute of Industrial Hygiene and Occupational Diseases, Prague, February 11, 1957.

Zusammenfassung

An Leberschnitten von Rattenweibchen wurde festgestellt, dass anorganisches Thiophosphat die Oxydation von Parathion zu Paraoxon hemmt. Dies hängt von den variierenden Konzentrationen des Substrates und des Inhibitors gemäss der antikompetitiven Hemmung ab.

Ein diuretisch wirksamer Stoff aus Wacholder (*Juniperus communis* L.)

Im Rahmen des Studiums von bewährten Heilpflanzen, welches die Isolierung von neuen biologisch aktiven Substanzen zum Ziele hat, befassten wir uns mit dem diuretischen Prinzip des Wacholders (*Juniperus communis* L.)¹. Einzelne Fraktionen des Wacholderbeeröles wurden auf ihre diuretische Wirkung geprüft.

Die Wirkung jeder Substanz wurde bei subkutaner Verabreichung an mindestens 12 weissen Ratten, denen vorher 2,5 ml physiologischer Kochsalzlösung verabfolgt worden war, festgestellt und mit der Diurese einer gleich starken Gruppe von Kontrolltieren verglichen. Zur Aus-

⁸ D. BURK, quoted by E. R. EBERSOLE, C. GUTTENTAG, and P. W. WILSON, Arch. Biochem. 3, 399 (1944). – J. B. SUMNER and K. MURBÄCK, The Enzymes (New York 1950).

¹ V. HEROUT, O. MOTL und F. ŠORM, Chem. listy 48, 589 (1954); Collection 19, 990 (1954). – O. MOTL, I. JANKŮ und H. RAŠKOVÁ, Čs. farm. 4, 240 (1955). – O. MOTL, V. HEROUT und F. ŠORM, Chem. listy 50, 128 (1956).

	Urinmenge als Prozentsatz verabfolgter Flüssigkeit			
	nach 4 h		nach 24 h	
	Durchschnitt	95%-Sicherheitsgrenzen	Durchschnitt	95%-Sicherheitsgrenzen
Kontrolle	14,3 ± 4,1	3,8–24,8	43,8 ± 4,6	32,0– 55,6
Wacholderbeeröl 1,0 ml/kg subkutan . .	44,0 ± 6,9	26,7–61,0	85,3 ± 7,6	65,8–104,8
Terpinenol-4 0,1 ml/kg subkutan . . .	78,4 ± 7,1	60,2–96,6	157,6 ± 7,1	113,7–201,5

wertung der Wirkung wurde die Menge ausgeschiedenen Urins, ausgedrückt als Prozentsatz verabfolgter Flüssigkeit, nach 4 und nach 24 h bestimmt.

Nach dem Vorversuche mit der Kohlenwasserstofffraktion keine besondere Wirkung zeigten, konzentrieren wir uns ausschliesslich auf die Prüfung der diuretischen Wirksamkeit der sauerstoffhaltigen Fraktion. Das darin enthaltene Terpinenol-4 (1-*p*-menthen-4-ol), Sdp. 89° bei 10 mm Hg, d_4^{20} 0,9259, n_D^{20} 1,4762, $[\alpha_D^{20}] + 28,1^\circ$ ² zeigte im Vergleich mit Wacholderbeeröl, eine gut ausgeprägte diuretische Wirkung (Tabelle).

Dem Terpinenol-4 muss ein grosser Teil der diuretischen Wirkung der Wacholderbeeren zugeschrieben werden.

Die Ergebnisse pharmakologischer Prüfungen und histologischer Untersuchungen nach langdauernder Verabreichung von Terpinenol-4 waren so befriedigend, dass mit der klinischen Prüfung der Substanz begonnen worden ist.

I. JANKŮ, M. HÁVA und
O. MOTL

Chemisches Institut der Tschechoslowakischen Akademie
der Wissenschaften, Prag, den 29. September 1956.

Summary

It has been demonstrated that terpinenol-4, contained in juniper-berry oil, has a marked diuretic effect. On the basis of this finding, it is assumed that this substance is the proper diuretic factor of juniper-berries.

² Ohne Lösungsmittel gemessen.

The Control of Ovary Development in Worker
Honeybees (*Apis mellifera*)

Introduction.—It is generally agreed that the ovaries of adequately nourished worker honeybees develop to some extent if the queens are removed from their colonies. For example, HESS¹ found that in nine out of eleven colonies the ovaries of 10% or more of the worker bees developed within a week of the removal of their mated queens.

It has also been shown that ovary development will occur in well-nourished queenless workers kept in small groups in cages at about 30°C, and that the presence of either a living or dead mated queen is sufficient to prevent such development under these conditions².

As a result of her experiments HESS¹ suggested that some material substance produced by the queen is circulated in food amongst her workers and is capable of

inhibiting the development of their ovaries. The existence of such an inhibitory substance (queen substance) has since been verified³, and biologically active extracts of it have been obtained from the bodies of queens in a number of organic solvents⁴.

In 1954 BUTLER⁵ found that bees who had just finished licking the body of their queen offered regurgitated food to other members of their colony more often than other bees of the same colony who had only just examined but not licked their queen. Furthermore, he was able to show that less ovary development occurred amongst a group of well-nourished, queenless worker honeybees to whose food had been added the honeystomach contents of bees who had just licked their queen than amongst a similar group of bees to whose food had been added the honeystomach contents of bees who had had no opportunity to lick a queen for several hours⁶. It thus appeared that queen substance was capable of inhibiting ovary development even when received by worker bees in their food rather than directly from their queen. VOOGD⁷, on the other hand, concluded from her own experiments that queen substance was effective *only* when the bees were able to lick it from some object, such as a dead worker bee.

Further experiments have now been made to compare directly the effectiveness of queen substance in inhibiting ovary development in worker bees when supplied to them by these two methods.

Experimental Method.—In July 1956, 216 newly emerged worker honeybees were taken from a normal colony which had made no attempts to swarm nor to supersede its mated, laying queen. The ovaries of 36 of these bees were immediately examined and found to be undeveloped. The remaining bees were divided at random into 5 groups of equal size. Each group of 36 bees was placed in a separate, well-ventilated, perspex cage, 60 mm × 5 mm × 95 mm high. A piece of empty worker brood comb, 40 mm × 40 mm, was fixed in the centre of one of the sides of each cage.

The bees in each cage were supplied continuously with distilled water and also with pollen/candy in separate feeders. The pollen/candy was prepared by mixing icing sugar with 20% by weight of fresh bee-collected pollen and sufficient distilled water to make a stiff paste. The cages of bees were kept in the dark in an incubator at approximately 32°C for 20 days.

An extract of queen substance was prepared as follows: 2 mated laying queens were killed by chilling them in a

¹ G. HESS, Beih. Schweiz. Bienenztg. 1, 33 (1942).
² J. PAIN, XI. International Beekeeping Congress, Copenhagen 1954. — A. P. DE GROOT and S. VOOGD, Exper. 10, 384 (1954).

³ A. P. DE GROOT and S. VOOGD, Exper. 10, 384 (1954). — S. VOOGD, Exper. 11, 181 (1955); 12, 199 (1956). — C. G. BUTLER, Proc. Roy. Ent. Soc. Lond. (A) 31, 12 (1956).
⁴ A. P. DE GROOT and S. VOOGD, Exper. 10, 384 (1954). — S. VOOGD, Exper. 11, 181 (1955); 12, 199 (1956).
⁵ C. G. BUTLER, Trans. Roy. Ent. Soc. Lond. 105, 11 (1954).
⁶ C. G. BUTLER, Proc. Roy. Ent. Soc. London 31, 12 (1956).
⁷ S. VOOGD, Exper. 11, 181 (1955); 12, 199 (1956).

Table I.—Effects of treatments on ovary development in worker honeybees

Cage No.	Treatment	No. bees with developed ovaries	No. bees with undeveloped ovaries	% bees with developed ovaries
1	None	28	2	93
2	Extracted queen . . .	25	9	74
3	Reimpregnated queen.	14	22	39
4	Acetone in food . . .	31	3	91
5	Extract in food . . .	22	10	69

refrigerator. Their bodies were then extracted for 3 h in 10 ml of acetone in a micro-Soxhlet apparatus. This extract of queen substance was subsequently reduced in volume to 2 ml by evaporation and divided into 2 equal parts.

One of the mated laying queens which had been extracted in this way for 3 h was attached to the centre of the comb in one cage. The second mated laying queen which had been similarly extracted was reimpregnated with 1 ml of the concentrated acetone extract obtained from the 2 queens and attached to the centre of the comb in a second cage. (Reimpregnation of this queen's body was achieved by repeatedly bathing it in the extract and allowing the solvent to evaporate at room temperature.) The second 1 ml of the concentrated extract was thoroughly mixed with the pollen/candy (volume 8 ml) in a third cage in which no queen was present. 1 ml of acetone was added to the pollen/candy (8 ml) in a fourth cage, in which there was also no queen. Nothing was added to the food of the bees in the remaining cage nor was a queen present.

After 20 days all the bees remaining alive were killed and the degree of development of their ovaries determined. Five degrees of ovary development similar to those used by HESS¹ were recognised: 'no development', 'possible very slight development', 'some development', 'strong development', 'very strong development'. Since in summer in an average, apparently normal colony headed by a mated laying queen worker bees with ovaries showing 'no development' and 'possible slight development' are regularly found, these two degrees of development were classified together as 'undeveloped' and the remaining three degrees of development were considered together as 'developed'. This classification was also employed by DE GROOT and VOOGD⁸.

Results.—The results obtained are shown in Tables I and II.

Discussion.—VOOGD⁹ showed that the ovaries of those queenless worker honeybees who licked an object which had been impregnated with an acetone extract of a queen honeybee developed significantly less ($P < 0.01$) than those of bees who did not lick the impregnated object; but, when an extract of a queen was added to their food the number of bees with developed ovaries was less obviously reduced although, if one uses a 'single-tail test' the difference which she observed is just on the 5% level (i.e. if one ignores the possibility of a true result in the 'wrong' direction). The results given in Table I in this paper show that an acetone extract of a mated queen, whether given in food or on the body of a dead, previously extracted, queen, inhibited the development

Table II.—Analysis of results in Table I²

Comparison of difference in number of bees with and without developed ovaries in cages	Value of P
1 and 2	—
1 and 3	< 0.001
1 and 4	—
1 and 5	< 0.04
2 and 3	< 0.01
2 and 4	—
2 and 5	—
3 and 4	< 0.001
3 and 5	< 0.03
4 and 5	< 0.05

of the ovaries of worker honeybees (cages 1 and 3, 4 and 5, 2 and 3, 3 and 4). Therefore, if the data of VOOGD⁹ and of BUTLER⁶, as well as those in the present paper, are considered together it seems that an extract of queen substance (obtained in acetone from a queen) taken orally by queenless well-nourished worker honeybees, either in food or from the body of a dead bee, does have a definite inhibitory influence on the development of their ovaries.

It appears, however, from comparison of the degree of ovary development of the bees in cages 3 and 5, that presentation to the bees of the extract on the body of a previously extracted queen (which they were seen to examine and lick frequently) was more effective in inhibiting ovary development by these worker bees than was the addition of an equal quantity of the same queen extract to the food of a similar group of bees. It is possible that this difference may have been due to the bees receiving more queen substance when it was offered on the body of a dead, extracted, queen than when it was offered in food, or that it may conceivably have been due to the bees receiving a psychological stimulus when they licked an object impregnated with queen substance. This latter possibility seems rather unlikely, however.

C. G. BUTLER

Bee Department, Rothamsted Experimental Station, Harpenden, Hertfordshire (England), January 31, 1957.

Zusammenfassung

Während Übereinstimmung darüber erreicht worden ist, dass die Arbeitsbienen eine ovarienhemmende Substanz von ihren Königinnen erhalten, ist es weder klar-gestellt worden, wie diese Substanz unter die Arbeits-bienen verteilt wird, noch ob die Königin eine psychi-sche Wirkung auf sie ausübt.

Wenn die Versuchsergebnisse von VOOGD⁹ und BUTLER⁶ sowie die Resultate dieser Arbeit zusammen betrachtet werden, scheint es klar, dass ein Extrakt dieser Substanz, wenn er an weisellose Arbeiterinnen verfüttert wird, eine deutliche Hemmwirkung auf die Ovarienentwicklung ausübt. Die Versuchsergebnisse deuten jedoch darauf hin, dass diese Substanz wirkungsvoller ist, wenn sie den Arbeitsbienen auf dem Körper einer toten Biene, oder dem Modell einer Biene, dargeboten wird, als wenn man sie dem Futter beimischt. Es scheint aber unwahrscheinlich, dass dies auf eine psychische Reizwirkung zu-rückzuführen ist¹⁰.

⁸ A. P. DE GROOT and S. VOOGD, *Exper.* 10, 384 (1954).
⁹ S. VOOGD, *Exper.* 12, 199 (1956).

¹⁰ D. B. CARLISLE and C. G. BUTLER, *Nature* 177, 276 (1956).

Nouveaux livres - Recensioni - Buchbesprechungen - Reviews

Über Kurven und Flächen in allgemeinen Räumen

Von PAUL FINSLER

XII und 160 Seiten

(Verlag Birkhäuser, Basel, Schweiz, 1951)
(Geb. Fr. 15.40)

In seiner berühmten Habilitationsschrift *Über die Hypothesen, welche der Geometrie zugrunde liegen*, formulierte B. RIEMANN erstmalig in der Geschichte der Mathematik den Begriff der Geometrie eines Raumes mit allgemeiner Massbestimmung. Zunächst wurde die Riemannsche Geometrie entwickelt, und es blieb PAUL FINSLER vorbehalten, die erste systematische Untersuchung von Räumen mit allgemeineren Massbestimmungen durchzuführen. Seine Ergebnisse fanden ihren Niederschlag in seiner Göttinger Dissertation aus dem Jahre 1918, um deren unveränderten Nachdruck es sich hier handelt. Während schon frühere Arbeiten, namentlich von BLISS, LANDSBERG und UNDERHILL, ähnliche Tendenzen in dieser Richtung aufzuweisen hatten, beschäftigten sich diese hauptsächlich mit geometrischen Interpretationen der Variationsrechnung, wobei stets der klassische euklidische Hintergrund sehr stark in Erscheinung trat. Gerade dieser Mangel wird bei FINSLER konsequent vermieden, indem er sich durch rein geometrische Gesichtspunkte leiten lässt. Auch er pflegt den Zusammenhang mit der Variationsrechnung, insbesondere aber mit den in diese Disziplin eingeführten geometrischen Methoden seines damaligen Lehrers CARATHÉODORY. So steht gerade die Indikatrix im Mittelpunkt der grundlegenden Betrachtungen über das Längen- und Winkelmass. Auf diesen Begriffen stützen sich dann die Untersuchungen über das Berührungsmass und die Krümmungen von Raumkurven. Diese Kurventheorie wird auf mehrdimensionale Flächen übertragen, und viele aus der Flächentheorie bekannte Sätze werden für den allgemeinen Fall bewiesen. Es ist leider nicht möglich, in einem kurzen Referat auf die Fülle der schönen Ergebnisse einzugehen; auch ist es wohl überflüssig, die so grosse Bedeutung der Finslerschen Dissertation hervorzuheben. Schon die von H. SCHUBERT hergestellte Literaturübersicht, welche dem Nachdruck beigelegt ist, bezeugt durch ihre zahlreichen Literaturangaben, wie intensiv man sich seitdem mit der heute sogenannten Finslerschen Geometrie beschäftigt hat. Es ist sicher bedauerlich, dass man in den späteren Entwicklungen dieser Geometrie oft weit von den Finslerschen Gedankengängen abgewichen ist, indem man sich durch die Einführung der Methoden der Tensorrechnung bemühte, möglichst eng an die Riemannsche Geometrie anzuknüpfen. Gerade deshalb ist es um so erfreulicher, dass dieser Nachdruck entstanden ist, und der Referent möchte sich folgender Schlussbemerkung aus dem Vorwort von A. OSTROWSKI wärmstens anschliessen: «Es ist aber sicher zu erwarten, dass heute, nachdem diese Arbeit nun allgemein zugänglich geworden ist, noch mannigfaltige Wirkungen von ihr ausstrahlen werden.»

H. RUND

The Meaning of Relativity

By ALBERT EINSTEIN

5th ed., 166 pages
(Princeton University Press, 1955)
(\$ 3.75)

Dieses kleine Buch enthält eine Vorlesung über spezielle und allgemeine Relativitätstheorie, die EINSTEIN 1921 in Princeton gehalten hat. In gedrängter Form, die immer aufs neue durch ihre Einfachheit und Anspruchslosigkeit besticht, hat er hier die physikalischen und mathematischen Grundgedanken seiner Theorie dargestellt.

Dem Buch ist als neuer Anhang (auf 30 Seiten) eine Darstellung der Verallgemeinerung beigegeben, die EINSTEIN seiner Theorie in den letzten Jahren gegeben hat. Er war der Meinung, dass diese Theorie die logisch einfachste mögliche Feldtheorie sei.

Den Abschluss bilden zwei allgemeine Erwägungen, die die Frage betreffen, ob die Feldtheorie die atomistische und quantisierte Struktur der Materie ("structure of reality") erfassen könne. Es scheint, dass selbst EINSTEIN hieran gezweifelt hat.

Das Buch, das wohl zum klassischen Bestand der physikalischen Literatur gehört, möchte ich gerade auch jüngeren Fachgenossen zur Lektüre empfehlen.

M. FIERZ

Gesetzmässigkeiten der Gestaltwandlung im Blütenbereich

Ihre Bedeutung für das Problem der Evolution

Von ERICH NELSON

302 Seiten, 689 Abbildungen auf 14 Tafeln
(Verlag E. Nelson, Chermex s. Montreux)
(Publiziert mit Unterstützung des Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung)

Ein Privatgelehrter meldet sich zum Wort mit einer umfangreichen vergleichend-morphologischen Arbeit über Gestalt- und Stellungsverhältnisse der Blütenorgane, besonders der Kronblätter und Staubgefässe. Die sorgfältig gezeichneten und gemalten Figuren zeugen von genauer Beobachtung.

Als Beispiel für die Art der Behandlung sei der Begriff der «Gruppenstellung» herausgegriffen. HIRMER hat gezeigt, wie auf der Grundlage von Spiralstellungen nach der Limitdivergenz von $137\frac{1}{2}^\circ$ beim Übergang zur Wirtel-Stellung die Glieder eines Zyklus gruppenweise genähert auftreten mit Bevorzugung der Fibonacci-Zahlen 2, 3, 5, 8, ... NELSON sieht in der Gruppenbildung die Folge eines ungleich starken Ausweichens jüngerer Organe vor den älteren. Diese «differenzierte Alternanz» wirkt sich nicht nur in der Zahl, sondern deutlich auch in der Gestalt der Glieder aus. Individuelle Variationen, wie die Unterschiede von Art zu Art, zeigen, wie die Gestaltwandlung in der Evolution gesetzmässig fortschreitet. Durch die Gruppenstellung wird dabei ein gestörtes physiologisches Gleichgewicht unvollkommen,

durch den Übergang zu absoluter Äquidistanz und Alternanz vollkommen wiederhergestellt.

NELSON nimmt an, dass sich die Kronblätter der Angiospermen phylogenetisch nicht von grünen Hochblättern ableiten lassen wie der Kelch, sondern dass die Kronblätter nachträglich durch partielles Sterilwerden aus Staubblättern entstanden seien. Wo dennoch Übergangsformen zwischen Kelch und Krone vorkommen, beruhen sie auf einem «Vorgang der Annäherung», auf einer sekundären gestaltlichen Angleichung eines Organes an ein anderes von organophyletisch heterogener Natur.

Der Morphologe betrachtet den «Phänotypus» der Pflanzen in seiner unmittelbar sichtbaren Veränderlichkeit. Der Genetiker schliesst aus seinen Experimenten auf den hinter dem Phänotypus verborgenen «Genotypus». Bei NELSON ist der «Genotypus» verstanden bald als ein erblich fixierter Phänotypus, bald als eine

«Reaktionsnorm». Das anschauliche Denken der vergleichenden Morphologie und das entwicklungsphysiologische Denken durchdringen sich, ohne dass ein Ausgleich der beiden Denkformen erreicht wird.

NELSON wagt sich auch an das schwierige Problem der Vererbung erworbener Eigenschaften. Seine Ausführungen beginnen mit dem Satz: «Gesetzmässigkeiten und Parallelentwicklung der Gestaltwandlung stehen im Widerspruch zu allen mit einem ‚Zufallsgeschehen‘ rechnenden Evolutionstheorien.» Der Referent würde lieber die Schärfe der Gegensätze mildern und fragen: Was für Argumente hat der Morphologe vorzubringen für ein Zusammenwirken zufälliger Mutationen mit gerichteten Wandlungen in der Phylogenie? Zu solcher Fragestellung bringt NELSON wertvolles Material. Seine Arbeit ist der Beachtung der Fachgelehrten zu empfehlen.

O. SCHÜEPP

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Influence of Baroreceptor Reflexes on the Reactivity of the Autonomic Nervous System*

By E. GELLHORN**

Although in nerve-muscle preparations changes in the state of activity of an autonomically innervated organ are known to result in fundamental alterations in the action of sympathetic and parasympathetic nerves no systematic attempt seems to have been made to induce changes in autonomic reactivity and balance either via reflexes or by direct alterations in central autonomic structures such as the hypothalamus. Some of the results obtained in the study of reflexly induced autonomic imbalances are summarized in this paper. Their implications for physiology, medicine, and neuropsychiatry will be discussed in a forthcoming monograph¹.

If in very lightly anesthetized cats the blood pressure was altered by the injection of hypotensive and hypertensive drugs (acetylcholine, mecholyl, histamine, and noradrenaline respectively) characteristic changes in the excitability of the autonomic system in general and of the hypothalamus in particular occurred. The fall of the blood pressure was found to be associated with an increased responsiveness of the sympathetic system to direct stimulation of the posterior hypothalamus and to reflex stimuli acting on the sympathetic system. Conversely, the parasympathetic reactivity was increased during the noradrenaline induced rise of the blood pressure². The increased responsiveness of a specific

division of the autonomic system was designated as 'tuning'. During the state of sympathetic tuning produced by the injection of hypotensive drugs the autonomic balance was shifted to the sympathetic side as evidenced by the contraction of the sympathetically innervated nictitating membrane and the acceleration of the heart rate. In sensitive animals the hypotension was accompanied also by a contraction of the denervated nictitating membrane signifying a sympathico-adrenal discharge³. On the other hand, the state of parasympathetic tuning was associated with parasympathetic discharges as evidenced by a slowing of the heart rate.

During the state of sympathetic tuning the autonomic nervous system showed not only an increased responsiveness to stimuli acting on the sympathetic system but a diminished responsiveness to parasympathetically acting stimuli.

Corresponding changes occurred in the state of parasympathetic tuning. Thus the sympathetic responsiveness of the hypothalamus as indicated by the height of contraction of the nictitating membrane on stimulation of the posterior hypothalamus was diminished during the state of parasympathetic tuning (i.e. during the rise of the blood pressure following the injection of noradrenaline) whereas the responsiveness to a parasympathetically acting stimulus was enhanced. The latter phenomenon is illustrated by the fact that the stimulation of the sciatic nerve or the hypothalamus with a square wave current of low frequency produced a much greater parasympathetic effect (slowing of the heart rate and/or fall of the blood pressure) in the state of parasympathetic tuning than under control conditions.

The changes in autonomic reactivity described thus far were easily demonstrated with near threshold or subthreshold stimuli. The experiments demonstrated the validity of the law of reciprocal innervation for states of sympathetic and parasympathetic tuning.

That the changes in blood pressure and not a specific drug action were responsible for the effects of 'tuning'

* Laboratory of Neurophysiology, University of Minnesota, Minneapolis.

** These studies were aided by a grant from the W. LOUIS and MAUD HILL Family Foundation.

¹ E. GELLHORN, *Autonomic Imbalance and the Hypothalamus* (University of Minnesota Press, Minneapolis 1957).

² Unpublished experiments with E. S. REDGATE.

³ E. GELLHORN, *Physiological Foundations of Neurology and Psychiatry* (University of Minnesota Press, Minneapolis 1953), p. 471.

was apparent from the observation that a fall of the blood pressure through bleeding and a rise of the blood pressure through the injection of dextran were associated with characteristic alterations in autonomic reactivity. On the basis of these experiments it may be said that a moderate hemorrhage leads to a reversible state of sympathetic tuning whereas the injection of dextran induces a state of parasympathetic tuning².

Since variations of the blood pressure regardless of the mechanism involved seemed to lead to characteristic changes in the reactivity of the autonomic nervous system it appeared likely that the baroreceptor reflexes of the sino-aortic area would be involved. This hypothesis was verified by the observation that the characteristic effects of autonomic tuning were greatly diminished or abolished by denervation of the sino-aortic areas. The tuning effects described in this paper are therefore regarded as the result of the action of baroreceptor reflexes on central autonomic structures.

In view of the fact that earlier investigations⁴ had demonstrated the decisive influence of the state of excitability of the hypothalamus on the action of acetylcholine, mecholyl and histamine and on the noradrenaline-induced reflex slowing of the heart rate it was thought advisable to investigate the action of these drugs and of the baroreceptor reflexes on the hypothalamus. Advantage was taken of the fact that changes in the excitability of the posterior hypothalamus are accompanied by corresponding changes in the intensity of the hypothalamic-cortical discharges influencing the activity in the whole cerebral cortex⁵.

Therefore alterations in the electrocorticogram may suggest changes in the intensity of the hypothalamic-cortical discharge related to the state of excitation of the posterior hypothalamus.

Several groups of experiments were performed. In one group the action of hypotensive drugs on the ECG was investigated before and after discrete lesions had been made in the posterior hypothalamus by high frequency currents⁶. These experiments showed that after unilateral hypothalamic lesions the degree of cortical excitation induced by hypotensive drugs was lessened on the ipsilateral side. In the second group, the action of hypo- and hypertensive drugs on the cerebral cortex was investigated before and after sino-aortic denervation⁷. Under control conditions acetylcholine caused an excitation of the cerebral cortex indicated by an increased asynchrony of cortical potentials and an increase in the integrated amplitude of the fast potentials⁸. Noradrenaline, however, caused the opposite effect: the cortical potentials became more synchronized and the integrated amplitude of the potentials was increased for the low frequencies only. The effects of hypo- and hypertensive drugs on the ECG were practically abolished by sino-aortic denervation.

⁴ E. GELLHORN and E. REDGATE, Arch. int. Pharmacodyn. 102, 162 (1955). – E. REDGATE and E. GELLHORN, Arch. int. Pharmacodyn. 102, 179 (1955). – E. GELLHORN, H. NAKAO, and E. REDGATE, J. Physiol. 131, 402 (1956). – E. REDGATE and E. GELLHORN, Arch. int. Pharmacodyn. 105, 199 (1956).

⁵ E. GELLHORN, *Physiological Foundations of Neurology and Psychiatry* (University of Minnesota Press, Minneapolis 1953); Arch. int. Pharmacodyn. 93, 434 (1953); EEG Clin. Neurophysiol. 5, 401 (1953). – W. KOELLA and E. GELLHORN, J. comp. Neurol. 100, 243 (1954). – E. GELLHORN, Brain 77, 401 (1954). – W. KOELLA and H. BALLIN, EEG Clin. Neurophysiol. 6, 629 (1954).

⁶ Unpublished experiments with E. B. SIGG.

⁷ H. NAKAO, H. M. BALLIN, and E. GELLHORN, EEG Clin. Neurophysiol. 8, 413 (1956).

⁸ In these experiments the ECG was studied with an Offner frequency analyser.

The conclusion drawn from these experiments that baroreceptor reflexes, modified by the changes in the intrasinus pressure following the injection of acetylcholine and noradrenaline, act on the posterior hypothalamus and thereby alter the hypothalamic-cortical discharge was borne out by a third group of experiments. The action of acetylcholine and noradrenaline was studied on the potentials of the posterior hypothalamus. It was found that acetylcholine caused an excitation of the hypothalamic potentials (increased asynchrony and recruitment) whereas noradrenaline had the opposite effect⁹.

In recent years numerous investigators have shown that protoveratrine excites the baroreceptors of the carotid sinus¹⁰. If the baroreceptor reflexes act not only on the medulla oblongata but also on the hypothalamus (and thereby on the cerebral cortex) as our experiments suggest, one should expect that the hypotensive action of protoveratrin would depend on the state of excitability of the hypothalamus. Experiments were performed, therefore, on the action of protoveratrine (0.0005 mg/kg intravenously) on the blood pressure of cats⁹. The mean fall of the blood pressure in the control group on administration of this drug was 37 mm Hg whereas following reduction of the excitability of the posterior hypothalamus through the intrahypothalamic injection of nembutal or high frequency coagulation of this area the mean fall of the blood pressure was 5.9 mm Hg.

Zusammenfassung

Fallen des Blutdruckes bewirkt sympathische, seine Steigerung hingegen parasympathische «Umstimmung». Erstere ist durch Sympathicotonie und erhöhte Reizbarkeit des sympathischen Systems, letztere durch entsprechende Änderungen des parasympathischen Systems charakterisiert. Diese Änderungen des autonomen Systems resultieren aus den Blutdruckzügler-Reflexen, deren Wirkung sich auch auf den Hypothalamus posterior und von dort auf die gesamte Hirnrinde erstreckt.

⁹ Unpublished experiments with H. M. BALLIN.

¹⁰ L. CALLIAUW, Arch. int. Pharmacodyn. 107, 75 (1956). – S. C. WANG, S. H. NGAI, and R. G. GROSSMAN, J. Pharmacol. exp. Therap. 113, 100 (1955). – G. MATTON, Arch. int. Pharmacodyn. 103, 13 (1955).

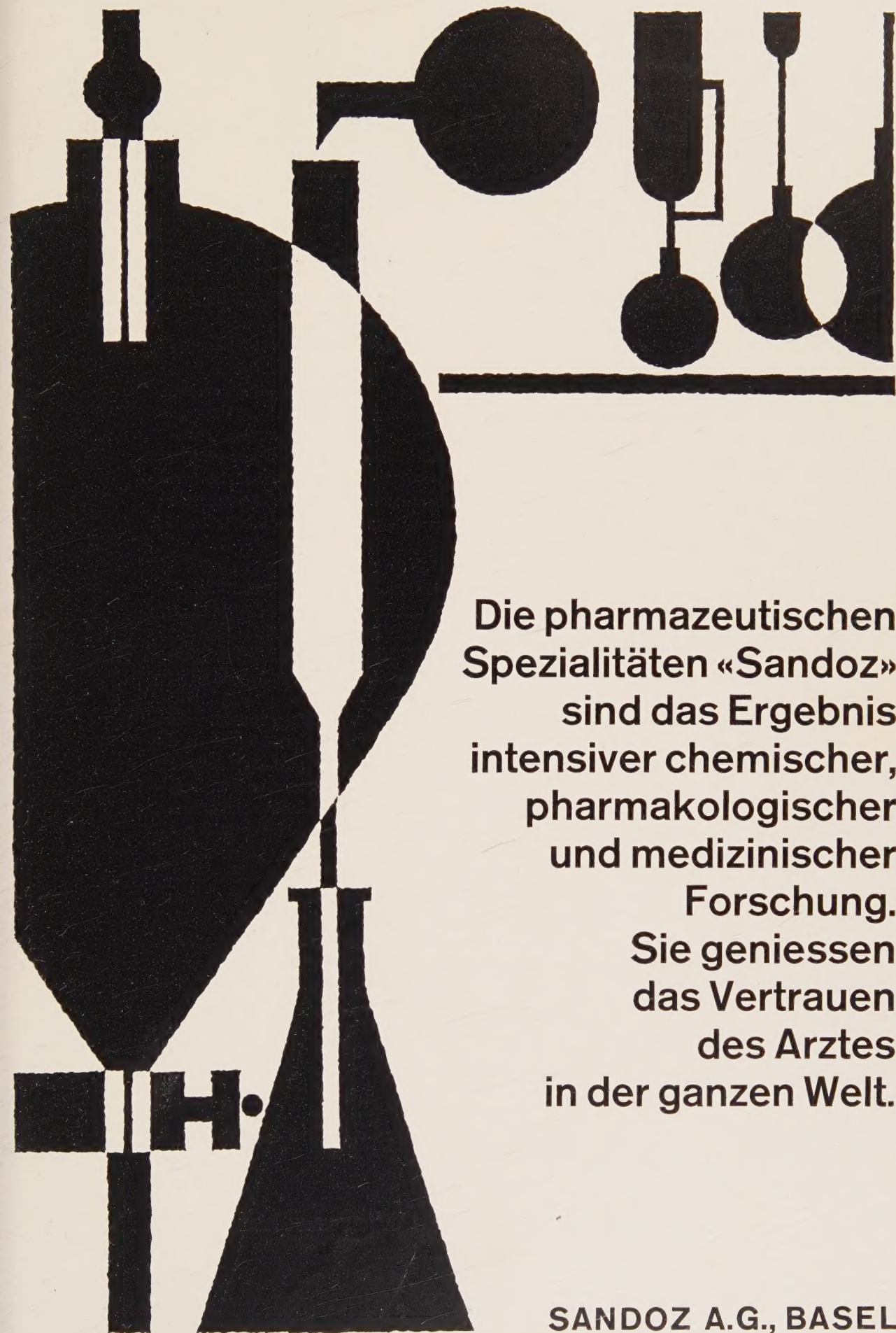
Corrigenda

H. BÄCHTOLD und A. PLETSCHER: *Einfluss von Isotrikotinsäurehydraziden auf den Verlauf der Körpertemperatur nach Reserpin, Monoaminen und Chlorpromazin*, Experientia, vol. XIII, Heft Nr. 4, S. 163 (1957).

Auf Seite 163, 11. Zeile von oben, soll es richtig heissen «*Rimifon*» (anstatt *Rimiform*).

MARIA SZÉKELY: *Die Bedeutung der Mitochondrienstruktur für die Zitronensäuresynthese*, Exper. 13, Heft Nr. 1, 24 (1957).

Auf Seite 25, linke Kolonne, nach der Tabelle III muss es richtig heissen: «Dabei konnte die Hemmung durch höhere Konzentrationen der an der Reaktion beteiligten Substrate und Coenzyme nicht aufgehoben werden» (anstatt aufgehoben werden).



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Tier- und Naturphotographie

Eine *neue* Zeitschrift, herausgegeben
von Professor Dr. FRITZ STEINIGER, Hannover

Ausgangspunkt einer Zeitschrift für Tier- und Naturphotographie

Bisher genügte es der Tier- und Pflanzenphotographie, ihren Gegenstand in einer möglichst ansprechenden Form darzustellen. Diese Aufgabe hat sie in ernster Arbeit heute bereits gelöst: Es gibt von allen wesentlichen Tieren und Pflanzen gute Aufnahmen, und wenn heute auch noch das eine oder andere zu ergänzen und zu erneuern ist, so kann man hier doch nicht eine vordringliche Aufgabe erkennen, wie sie sich die beginnende Naturphotographie vor einem halben Jahrhundert stellte. Was heute zum Teil noch fehlt, ist die pädagogisch geschickte Darstellung von *Tier- und Pflanze in ihrem natürlichen Lebensraum*. Denn der erfahrene Biologe wird zugeben, dass er nur zu oft Pflanzen und Tiere nicht so sehr nach Form und Farbe, sondern – oft unbewusst – nach der Art anspricht, in der sie sich in die Landschaft einpassen.

Auch die junge *tierpsychologische Wissenschaft und die Verhaltensforschung* am Tier bedürfen noch weitergehend einer photographischen Unterstützung. Es fehlt an guten Lichtbild-Darstellungen vieler tierischer Verhaltensweisen, ja selbst die Methodik dafür scheint bis jetzt noch nicht ausreichend entwickelt zu sein. Hier liegen noch beachtliche Aufgaben der Freiland-Tierphotographie vor, die anscheinend viel mehr Möglichkeiten hat, Unterlagen für die Verhaltensforschung zu sammeln, als es dieser Wissenschaftszweig bisher anerkennt.

Die beginnende Tier-Lichtbilderei stand vor einem halben Jahrhundert im Zeichen des gleichzeitig mit ihr beginnenden *Naturschutzes*. In dieser Verbindung sind sich bis heute beide im grossen und ganzen treugeblieben, wenn auch gelegentlich einmal der Betreuer eines Naturschutzgebietes einen Photographen hinausbefördern muss, der mit zu wenig Verständnis für Tiere und Pflanzen in das Gebiet eindrang. Der wirkliche Naturphotograph muss gleichzeitig Naturschützer sein und sich nach den Umgangsformen des Naturschutzes richten.

Noch nicht ausgenutzt sind die Möglichkeiten der Tier- und Pflanzenphotographie im *Biologie-Unterricht*. Der Biologielehrer, gleich welcher Stellung, sollte Naturlichtbildner sein. Denn nichts kann den Biologie-Unterricht so beleben und erleichtern, wie gute eigene Aufnahmen, die auch das Persönliche des Lehrers, ohne dass der Unterricht schablonenhaft wird, stark hervortreten lassen. Jemand, der den Biologie-Unterricht mit eigenen Bildern belebt, zeigt damit schon dem einfach denkenden Schüler, dass er sich mit dem Gegenstand des Unterrichtes stark beschäftigt hat, und dass er nicht etwa von Dingen berichtet, die ihm selbst kaum geläufig sind. Und auch für den mehr erwachsenen Schüler ist die Tier- und Pflanzenphotographie sehr zu empfehlen, mehr als das Sammeln von Dingen der Natur, das – auf breiter Ebene propagiert – doch manche seltene Art zum Erlöschen bringen kann.

Die Zeitschrift erscheint im Format 17 × 24 cm, zwanglos, ein Band = 6 Hefte kostet ca. Fr. 18.–. Wir erbitten Ihre Bestellung.

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